Investigations on the Development of Trypanosomes in Tsetse-Flies and other Diptera.

By

E. A. Minchin,

Professor of Protozoology in the University of London.

With Plates 8—13 and two Text-figures.

Contents.

I. Introductory.

In this memoir I propose to give a full account of the work done and the results obtained by me during my stay in Entebbe, Uganda, as a member of the Sleeping Sickness Commission from the beginning of April to the beginning of

December, 1905. Some of my results have already appeared in the form of preliminary reports; but with regard to some few points further study necessitates revision or correction of statements previously made. In my eight months in Uganda I accumulated a great deal of material which it has required much time and labour to work out. The study of this material has occupied the time left from other duties since my return from Uganda, and may now be considered complete.

The commission with which I went to Uganda was to study the life-cycle of Trypanosoma gambiense in its relation to the local species of tsetse-fly, Glossina palpalis. In view of the well-known life-cycle of the malarial parasite, as well as of the remarkable development described by Schaudinn (**40**), for the trypanosomes of the little owl (Athene noctua), it seemed probable that a study of the sleeping sickness trypanosome in its passage through the tsetse-fly would reveal an interesting developmental cycle. Unfortunately, these expectations have not been fulfilled, and, so far as the development of Trypanosoma gambiense is concerned, my investigations have been barren of results, and have yielded conclusions for the most part of a negative character. Incidentally, however, I have made some observations which are, perhaps, not without interest, on another species of trypanosome occurring in the fly, and a record of my work and its results may be of some use if only as a guide to future investigators in this field to enable them to avoid my failure.

In the exposition of the numerous and complicated data furnished by an investigation of this kind, it is difficult to steer clear between, on the one hand, too much subjective interpretation, which may become misleading; and, on the other hand, excessive elaboration of detail, which becomes tedious and difficult to follow. I propose, therefore, to divide this memoir into two chief parts: I shall first set forth the development, so far as I have observed it, of the two species of trypanosomes in a connected manner, and shall discuss the general question of the transmission of trypano-

some infections; after which I shall give a bare record, in chronological sequence, of my experiments and observations. The latter part is intended rather for reference, and to avoid the possible danger of omitting something which in the present state of our knowledge seems immaterial, but which may prove in the future of greater importance.

Before proceeding to describe my results it may be permitted to me to enter upon a brief personal explanation of my relation to the work of the Commission; after which I shall say a few words upon the methods of investigation employed.

When I came to Entebbe, at the beginning of April, 1905, my two colleagues, Lieutenant A. C. H. Gray, R.A.M.C., and the late Lieutenant F. M. G. Tulloch, R.A.M.C., were already at work upon the subject which I was sent out to investigate, and had discovered some facts of importance. In particular, they had found and studied the vast swarms of trypanosomes which are frequently found in the alimentary canal of freshly-caught tsetse-flies. Neither my colleagues nor myself had at that time any doubt but that these "wild" trypanosomes represented stages of T. gambiense. Since it was arranged that Gray and Tulloch were to work in connection with me, and since I did not wish to absorb, as it were, into our joint work anything of which the credit belonged to them independently, I requested them to write up and publish all that they had found before my arrival, so that we could start our collaboration on a clear footing. This they did, and the result was the memoir (17) published in the 'Sleeping Sickness Reports,' a memoir in which Tulloch's excellent drawings were done scanty justice in the reproduction. While Gray and Tulloch were engaged upon this report I commenced a systematic investigation of the anatomy of Glossina palpalis, then an untrodden field of study, upon which a preliminary report was published in the 'Proceedings of the Royal Society' (27). One of the first results of my dissection of the fly was to show that the structure termed by Gray and Tulloch in their memoir "the salivary gland" was really the

proventriculus, although I myself fell into the error of calling it the stomach in my communication.

Our further studies were divided between us as follows: Gray and Tulloch continued the investigation of the "wild" trypanosomes. I confined myself to a study of the changes undergone by T. gambiense when taken up by the tsetse-fly from infected animals. My intention was to work the development of T. gambiense from the beginning, so to speak, up to the condition found by Gray and Tulloch in the fresh-caught flies, and so link my researches on to theirs, with hope of thus getting the complete cycle. This expectation was not fulfilled, since more extended knowledge forced the conclusion slowly, but irresistibly, upon us, that the "wild" trypanosomes had nothing whatever to do with T. gambiense, but represented at least two distinct species occurring in the fly quite independently of sleeping sickness. As a result, however, of our subdivision of the work, my two colleagues accumulated an abundant material of the "wild" trypanosomes; my material of these interesting forms consists only of those found incidentally in flies used for experiments with T. gambiense, in all six flies having been found by me thus infected.

I returned to England at the end of 1905, leaving Gray and Tulloch still at work upon these problems. Our ever increasing doubts as to the true nature of the wild trypanosomes led to our planning a number of crucial experiments, which were carried out by Gray and Tulloch on the island of Kimmi after my departure. The result was to demonstrate conclusively the distinctness of the "wild" trypanosomes from T. gambiense. Meanwhile I had written, shortly after my return to England, an interim report on my work at Entebbe, for the information of the Tropical Diseases Committee of the Royal Society. This report was also utilised by me in my candidature for the chair of Protozoology, which I now hold, and a printed copy of it was sent by me to my friend M. Mesnil, of the Pasteur Institute, who, not being aware of its private nature, of which he had not been

informed, inserted an abstract of it in the 'Bull. Inst. Pasteur.' I am indebted to M. Mesnil's happy indiscretion for securing to me the priority of the statement, that the "wild" trypanosomes of Glossina palpalis were quite distinct from those of sleeping sickness—a conclusion to which Novy came independently after studying preparations sent him by Gray; his results were published in a memoir (32), in which he named one of the wild species T. grayi.

Shortly after this came the appalling news that our colleague, Tulloch, had become infected with trypanosomes. This most sad event, of course, put an end to further work, and Gray returned to England, bringing Tulloch with him. We then wrote a preliminary account of our results (29), which, so far as poor Tulloch was concerned, was a posthumous work when published. Our collaboration then broke up, and I began the detailed study of my material, and discovered the encystment of T. grayi, a result which I at once communicated to the Royal Society (28). It is a common human weakness to attribute failures to bad luck; nevertheless I cannot but deeply regret that I did not discover the encystment while I was in Entebbe. Had I done so, it would have modified the whole course of my researches. The fly in which this discovery was made was one of the last examined by me, a fortnight before I left off working. As I was then professor at University College, however, it was imperative upon me to return to my duties, having already had my leave of absence generously extended by the College for an extra three months.

Such is, in brief, the history of the conditions under which these investigations were carried on. It only remains for me to perform the pleasant duty of thanking those who have helped in my task. To the Tropical Diseases Committee of the Royal Society, in the first place, I am indebted not only for sending me to Uganda, but for providing me with assistance in working through my material after my return. Without the help and experienced advice of my colleagues on the Commission, Gray and Tulloch, I could have done little.

Since my return to England, my two assistants, Dr. J. D. Thomson and Dr. H. M. Woodcock, have rendered me much service in various ways, and Miss E. Y. Thomson has been of great assistance in the tedious task of searching through blood-films for trypanosomes and making counts of them. To each and all of these I desire to express my obligations and return my best thanks.

Methods of investigation.—I commenced my work, as I have said, by making a study of anatomy of Glossina palpalis. In undertaking this I was influenced largely by Schaudinn's work (**40**) on Trypanosoma noctuæ, in which he describes extensive migrations of the trypanosomes in the body of the invertebrate host. In tsetse-flies, however, I never found trypanosomes outside the alimentary canal, in spite of much searching; in this point my results agree with those of Stuhlmann (**41**).

My report on the anatomy of the fly (**27**) contained some errors which I desire to correct; they have been corrected in the reprint in the Reports of the Sleeping Sickness Commission. All through I used the term stomach for what should have been called the proventriculus; in this memoir I shall use the latter term. The true stomach in Glossina is represented by the first part of the digestive tract in the abdomen, that is to say, by the coils which in my figures and description were numbered 5, 6, 7. It is this region which becomes congested with blood after feeding, and in which the blood retains its bright red colour. From the stomach the blood passes into the next coils of the digestive tract, which constitute the true intestine, and here it becomes blackish in colour. Hence we may conveniently speak of the red blood, meaning that in the stomach, and the black blood, meaning that in the intestine proper. The red blood is thick and jelly-like, very difficult to smear out unless broken up with salt-citrate solution[1]; the black blood, on the contrary, is very fluid and watery, the number of corpuscles is more or

[1] Made up as recommended by Laveran and Mesnil (**23**); ·5 gr. sodium chlorine + ·5 gr. sodium citrate + 100 cc. H_2O.

less diminished, and there are usually numerous square crystals in it. The black blood stops short at the point where the Malpighian tubules arise, and where the proctodæum commences. The proctodæum contains no blood, but a yellowish fluid in which are suspended numerous coarse granules showing Brownian movement. Hence in a fly about twenty-four hours after feeding the three regions of the abdominal gut, namely, stomach, intestine, and proctodæum, are marked out by their respective colour—red, black, and pale yellow—in a way to make them easily discernible with the naked eye.

In the examination of a tsetse-fly for trypanosomes I usually began by inserting a fine capillary tube into the pericardial space, in order to draw up some of the fluid circulating in the hæmocœle. It was usually possible to draw up a small drop of the fluid. Some flies, however, were very anæmic and dry; such flies always proved on further examination to be sickly, and usually contained great numbers of bacteria in the gut, in which no blood as a rule was found. The hæmocœle fluid contained always peculiar amœboid corpuscles, generally more or less fusiform, with each end prolonged into a pseudopodium-like process; sometimes one end would show two such processes. The corpuscles showed distinct changes of form. No trypanosomes, however, were found in the hæmocœle. The next step would be the removal of the dorsal integument of the abdomen, and the examination of the contents of the salivary glands and the Malpighian tubules. The salivary glands contain numerous corpuscles or spherules of circular contour, varying from about 9 to 15μ in diameter, imbedded in a granular matrix. Each corpuscle contains an irregular spot towards the centre, usually angular in shape and looking like a split or space. With picrocarmine the corpuscle takes a pink tinge and the central spot a deeper colour. In the fresh condition the salivary corpuscles are about the same size as the fat-globules liberated from the fat-body, but distinguishable from them by their less refringent appearance and also

by the fact that the fat-globules float upwards, while the salivary corpuscles lie at the lowest focus. The Malpighian tubules contain coarse, yellowish-brown granules similar to those found in the proctodæum. Neither in the salivary glands nor in the Malpighian tubules did I ever find trypanosomes or any bodies other than those already mentioned. As I have already stated, the statement of Gray and Tulloch (**17**), to the effect that they found trypanosomes in the salivary gland must be corrected; I was present when they made the observation, and saw the preparation; by salivary gland was meant proventriculus.

After the Malpighian tubules I next examined the genitalia, either testes and seminal vesicles, or ovaries, receptacula, and oviducts (including uterus and embryo, if present). Here also I found in no case anything but the proper contents of these organs, which I need not describe in detail.

The last process is to dissect out the gut for its whole length and to divide it into its four regions, namely (1) the proventriculus with the thoracic intestine and sucking stomach, (2) the stomach with the red blood, (3) the intestine with the black blood, and (4) the proctodæum and rectum. In none of the flies dissected by me did I find trypanosomes in the proventriculus, but Gray and Tulloch, with their more abundant material of T. grayi and T. tullochii, frequently found these species in this part. No trypanosomes were ever found in the sucking stomach,[1] which in the normal condition is filled with air, but which immediately after feeding is found to contain traces of blood, and is sometimes quite full of blood, though this is a rare condition, and perhaps the result of some functional derangement of the organ. The fly, when feeding, appears to fill the sucking stomach with blood, and then to expel it thence into the digestive tract. It can be observed that when sucking blood the fly at intervals raises its head up a little, thus

[1] Gray and Greig (**18**) report trypanosomes in the "ventral food reservoir" (sucking stomach?) twelve hours after feeding. Stuhlmann (**41**) prefers the name "crop" for the sucking stomach.

partially withdrawing the proboscis from the skin of its victim, and then lowers it again.[1] Probably when the proboscis is lowered the sucking stomach is being filled, and when it is raised the blood is being expelled from the sucking stomach. This would account for the traces of blood found in the sucking stomach, and also in the proventriculus and thoracic intestine, after a meal. If the fly over-fed itself, it might not be able to discharge the last bolus of blood from the sucking stomach.

When the digestive tract is gorged with blood it is so distended, and the wall is so thin, that it is a difficult matter to dissect it out without rupturing it and letting blood escape. My colleagues, Gray and Tulloch, taught me a simple and effective method for doing this operation, which is as follows: The terminal segment of the abdomen is snipped off, the body is laid flat on an ordinary slide, and then a mounted needle, seeker, or other suitable instrument, is pressed down flat on the waist or base of the abdomen and passed along with an even, steady pressure towards the tip of the abdomen, so as to squeeze all the contents of the abdomen out on to the slide. With a little care and practice all the abdominal organs can be squeezed out quite uninjured, and can be separated from one another on the slide afterwards. In most cases the thoracic intestine and proventriculus are pulled out together with the abdominal contents. This simple method is most useful for rapid examination of a number of flies.

In order to make smears of the stomach-blood, it is necessary, as already stated, to mash it up with a little salt-citrate solution; for the intestinal and proctodæal contents this is not necessary. It is best to avoid, as much as possible, the use of salt-solutions. By comparing preparations made from pure blood with those of blood that had been mixed with citrate solution, I found the trypanosomes distinctly altered in form in the latter. Our method was to draw up the intestinal

[1] "Der Rüssel wird . . . während des Saugens häufig sägend auf- und abbewegt," Stuhlmann (**41**, p. 4). My statements in the text were written before Stuhlmann's memoir came to hand.

contents into capillary glass tubes, from which fine drops were expelled by blowing them gently on to slides, in order to make the smears. When made, the smears were sometimes dried rapidly in the air and then fixed with absolute alcohol or methyl alcohol, sometimes fixed with osmic vapour, employed in the manner recommended to me by my friend Dr. Plimmer: twenty drops of 4 per cent. osmic acid, with one drop of glacial acetic, placed in a stoppered tube of sufficient calibre to hold a slide, and the wet smear placed into the tube for about half a minute. I also tried a modification of this, as follows: A drop of blood on a slide was exposed, as rapidly as possible, to the osmic-acetic vapour for about half a minute, then mixed with an equal-sized drop of equal parts of fresh serum and dilute glycerine, and the whole smeared out. Subsequently the smear was fixed with absolute alcohol, without letting it dry; the glycerine was used in order to keep it moist for any length of time. I found, however, that this method tended to shrink the trypanosomes, though successful in other ways. It would be necessary to experiment in order to find the exact proportion of glycerine that should be mixed with the serum. In general I found the form of the body much more perfectly preserved in osmic-fixed smears than in air-dried preparations; the trypanosomes appear solid and plastic in the former, and always more or less flattened out in the latter. It seems, indeed, inevitable that the violent method of drying must deform a soft protoplasmic body.[1]

I wasted, I am sorry to say, a great deal of precious time trying to stain trypanosomes by the ordinary technique, which gives such good results with other Protozoa, especially the various carmine and logwood stains, which all proved useless. It is a puzzle to me why the Protozoa parasitic in blood should be so entirely different from other Protozoa in their staining reactions. I have always experienced just the same difficulty in trying to stain other Protozoa by the methods so successful for blood parasites. I fell back, finally, entirely upon the

[1] Lühe (26, p. 70), makes some valuable remarks on this point. Compare also Plimmer (35).

classic Romanowsky stain, using either Leishman's method, Laveran's Bleu-Borrel method, or Giemsa's stain. Latterly I used Giemsa's stain entirely, differentiating with tannin-orange solution (Unna's, obtained from Grübler). As a rule the smears were "refreshed" with fresh blood-serum, after the procedure recommended by Leishman. I kept my smears uncovered. Those that have been much used for study are now, I find, deteriorating, but those that have not been much looked at seem to be quite unaltered. Frequent baths of cedarwood oil and xylol alternately effect the stain after a time. Some smears, owing to pressure of time, were left unfixed, and were fixed after my return; none of these were very good.

Parasites of Glossina palpalis.—I examined the contents of the digestive tract and other organs in freshly-caught hungry flies with the object of making myself acquainted with the native parasites, if any, of the fly, in order to avoid confusing with them the stages of trypanosomes. Except for bacteria, however, the fly was very free from internal parasites. No gregarines or other sporozoa were found; it would, indeed, be improbable that a tsetse-fly, which apparently feeds exclusively on the blood of vertebrates, should acquire an infection of such parasites, which, as a general rule, are taken up with food in an encysted form by their hosts.

The commonest object in the gut was a large bacillus (figs. 111–123), apparently always present, sometimes in enormous numbers, especially in the stomach. When plentiful they occurred in masses or bundles; in such cases the flies always appeared sickly, emaciated, and anæmic, and usually had the digestive tract empty of blood, even when they had been put on to an animal; whether through the fly refusing to feed, or through rapid absorption of the blood by the bacteria, was doubtful. These bacteria show, apparently, only Brownian movement.[1]

[1] Stuhlmann (41, pp. 38, 39), has seen these or similar bodies in G. fusca. He believes them to be protozoa and not bacteria, and considers that they are not parasites because they are found to be invariably present. I cannot agree with him in either of these conclusions.

In the proctodæum and hinder part of the intestine I noted "sausage-shaped protoplasmic bodies, apparently flagellate, swimming actively" (figs. 124, 125). These were perhaps a form of the bacterium already described, as I noted others that appeared to be intermediate between the two forms.

In the same regions I noted also in one fly slender thread-like organisms, wriggling actively; perhaps spirochætes. I never found them, however, in my smears. In one fly (batch of Oct. 1st, 1905, examined Oct. 5th) I noted the red blood in the stomach "swarming with small motile bacteria, not the large inert forms ordinarily found." This fly was one bred out in the laboratory.

I frequently found, in the blood taken from tsetse-flies, and in my smears, curious bodies apparently of vegetable nature; figs. 127–131 show rough sketches from life of these bodies; fig. 126 is from a smear, drawn with the camera lucida, magnified 2000 linear. As may be seen, they are more or less spindle-shaped bodies, sometimes enclosed by a distinct membrane, sometimes not; the contents are divided into two, four, or more cells, containing each one or two nuclei. The nuclei sometimes appear in the living condition as clearer spaces, sometimes as darker spots. The colour of these bodies in life is greyish with a slightly greenish or brownish tinge. My notion of these bodies was that they came really from the solutions of salt or sodium citrate used in the dissections. Quite recently examining the blood of a rudd (Leuciscus erythrophthalmus) for trypanosomes, in Norfolk, I was surprised to find a quite typical example of these organisms. In this case the blood, in which no trypanosomes could be found, had been taken from the heart of a fish by means of a capillary glass tube, which had been previously washed through with normal saline solution. Hence there was a possibility that the body seen might have come from the salt solution. It may, however, be some form of organism inhabiting blood. I content myself with noting the occurrence of these bodies fairly frequently in tsetse-fly preparations.

In one fly I noted oval, refringent, motionless bodies in the proctodæum. In the sucking stomach I noted, in the wall, "long, greenish-yellow filaments, consisting each of a row of joints of varying lengths."[1]

Nomenclature of the structural parts of trypanosomes.—There is much diversity in the names applied by different authors to some parts of the trypanosome body, more especially with regard to the smaller chromatic nucleus, and also in the use of the terms anterior and posterior. The smaller nucleus is termed usually in England the micronucleus; in France the centrosome; and in Germany the blepharoplast. Each of these terms is open to objection, the term micronucleus, in my opinion, least of all, so long as it is used in a purely descriptive sense to mean simply a small nucleus; the danger is, however, that it leads to instituting a comparison with the micronucleus of Ciliata, which is a totally different structure, a reserve generative nucleus. Whatever the nature of the micronucleus of a trypanosome may be, there is no evidence that it is composed solely of generative chromatin.

The terms blepharoplast and centrosome raise the whole question of the nature of the bodies so named—a question upon which it is possible to have much difference of opinion. It is enough for me to refer here to the recent memoir by Goldschmidt and Popoff (**16**), in which the subject is discussed at length, and to state my own views: I regard a centrosome as an achromatic body, in connection with a nucleus; and a blepharoplast as a body of the same nature as a centrosome, but in connection with a protoplasmic locomotor apparatus, such as a flagellum or cilium. This is the sense in which I understand these terms; others may differ from me.

With regard to trypanosomes, I may point out, in the first place, that the "micronucleus" is certainly a chromatic body and cannot be classed with achromatic structures. Apart from its staining reactions, which are more intense than those of the

[1] Compare Stuhlmann (**41**, p. 47).

larger nucleus, I may cite the observations of Schaudinn (**40**), who describes it as arising in Trypanosoma noctuæ by an unequal division of the zygote nucleus; and I may further draw attention to the condition in Trypanoplasma, where it is as large as, or even larger than, the other nucleus, so that the term micronucleus for it becomes rather a misnomer.

In the second place, I may point out, as others have done also, that the flagellum does not arise from the smaller nucleus, but quite independently of it, from a minute basal granule.[1] The behaviour of the flagellum and of the smaller nucleus in division also shows clearly their complete structural independence, as I have pointed out below (p. 193).

I consider the basal granule of the flagellum as a true blepharoplast in the sense in which I have defined the term above; and I regard the nuclear apparatus of trypanosomes as specialised into two distinct portions, one regulating the function of locomotion, the other that of nutrition. Hence I consider that the terms kinetonucleus and trophonucleus, suggested by Woodcock (**42**), express most correctly the true nature of these bodies, and I shall employ these terms in my descriptions, though it is often convenient to speak of the trophonucleus simply as the nucleus, and as a further abbreviation I shall sometimes refer to the two bodies as *n* and *N* simply.

From the true blepharoplast arises the flagellum, which passes to the surface of the body and runs along the edge of the undulating membrane as the marginal flagellum, until it reaches the end of the body, where it becomes a free flagellum of greater or shorter length. According to Schaudinn's observations on the formation of the locomotion apparatus in Trypanosoma noctuæ (**40**), there exists also a distal blepharoplast, as it may be termed, situated at the

[1] Dutton, Todd, and Hannington (**15**, p. 219) point out that "the thickened end of the undulating membrane ends not in it [*n*] but in a pinkish basal granule or 'diplosome'" (why "diplosome"? It is only double when about to divide). Compare Novy (**33**), pp. 5 and 6.

free end of the flagellum. It is stated that the flagellum and undulating membrane are formed from the achromatic apparatus of a nucleus spindle, of which the central spindle gives rise to the flagellum and the mantle fibres to the myoneme-fibres of the undulating membrane, while the two centrosomes become the blepharoplast. These statements have received some confirmation from the observations of Robertson (**37**) on the formation of the flagellum in the trypanosome of Pontobdella muricata.

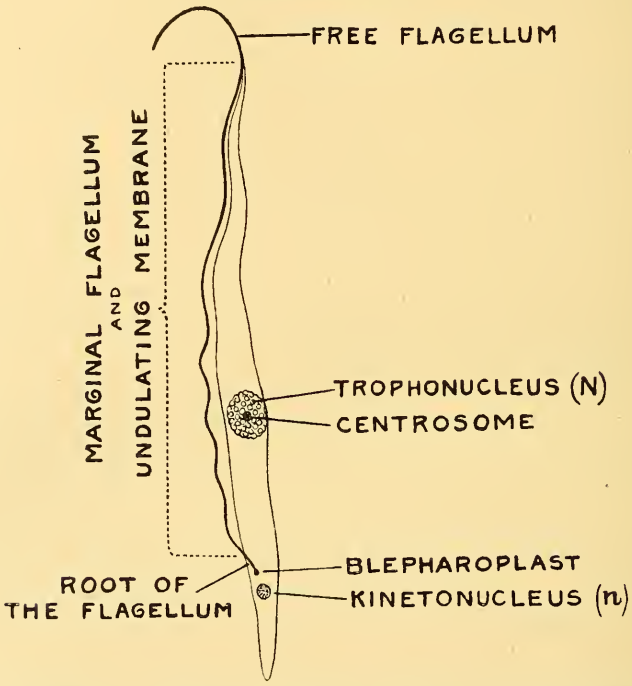
I have frequently noticed, especially in the slender forms of T. grayi, that the flagellum is distinctly thickened at the free extremity, but I should not like to affirm the existence of a definite blepharoplast at this point. Prowazek (**36**) has also described for T. lewisi a complicated arrangement of anchoring granules and fibrils. I can only say that in my material I have not seen them.

With regard to the use of the terms anterior and posterior much has been written, and it has been pointed out by Woodcock (**42**) and Lühe (**26**) that what is morphologically anterior in one trypanosome may be posterior in another. It cannot, I think, be disputed that there may be two entirely different lines of phylogenetic evolution amongst the organisms grouped generally as trypanosomes (see below, p. 219), but in the present state of knowledge it is not possible to state definitely which of the two possible modes of orientation is applicable to a given species. Hence it would be best, perhaps, to avoid altogether the use of the terms anterior and posterior in describing trypanosomes, and to speak of the flagellar and anti-flagellar extremities, but such a terminology becomes very cumbrous in practice. I shall speak of the flagellar extremity as anterior, the anti-flagellar as posterior, using the terms in a purely descriptive sense and without prejudice to the morphological questions involved. So far as I have observed, when trypanosomes are moving freely they travel usually with the flagellar extremity directed forwards, but when pushing their way amongst blood-corpuscles they do so with the free flagellum of the undulating

membrane directed backwards, their movement in the latter case being similar to that of a Trypanoplasma.

The accompanying diagram (Text-fig. A) is intended to

Text-fig. A.



make clear the terms and the use which I shall make of them in this memoir.[1]

II. Observations on Trypanosoma gambiense.

(A) T. gambiense in the Blood of the Vertebrate Host.

My observations relate chiefly to T. gambiense in the blood of monkeys, all of which had been infected, directly or indirectly, by inoculation with cerebro-spinal fluid from sleeping sickness patients, with the exception of one, which became infected by the bites of tsetse-flies caught in the

[1] See also Appendix (p. 252).

neighbourhood of Entebbe; I shall refer to this monkey briefly as the fresh-fly monkey. I have also preparations of cerebro-spinal fluid from patients, and of the blood of a chimpanzee, eighteen days after inoculation with human cerebro-spinal fluid. I have no preparations of human blood showing trypanosomes.

In all cases alike the trypanosomes are distinguishable by their general build and appearance into slender, stout, and intermediate forms.[1] In the living condition the slender forms appear more snake-like, and are active in their movements, while the stout forms are fish-like in shape, and less motile. In successful preparations of blood-smears the three forms can usually be distinguished readily, especially in films fixed with osmic acid vapour, which preserves the body-form very perfectly. In films preserved by the ordinary drying method the different forms appear less differentiated. This is evidently the result of a slight flattening-out of the body, produced by the method of drying. The two extremes of the series—namely, the stout and slender types—contrast with each other, not only in their proportions, but also in the relative length of the free flagellum, which is long in the slender forms, short in the stout ones. The trypanosomes of the intermediate type can also be subdivided into two types by this character, so that all the trypanosomes in a given blood-preparation can be divided into two classes—those with long and those with short flagellum. This character, which is plainly seen in the figures of Bruce and Nabarro (5), is more reliable for use as a morphological distinction than the stoutness of the body—a character liable to alteration or deformation due to imperfect preservation.

The extreme of the stout type is seen in the so-called

[1] Moore and Breinl (30) state that they are unable to distinguish any marked dimorphism, and that the so-called males and females are "arbitrarily chosen" extremes in a continuous series. It was never pretended that they were anything else than the extreme differentiations, obvious naturally, of a neutral or intermediate type; as will be described below, after twenty-four hours in the invertebrate host the intermediate forms disappear, and only the extremes remain, arbitrarily selected or differentiated by the action of their environment.

"stumpy" forms (figs. 9, 10, 17, 20, 21, 33–35), in which the body is short and stout, usually rounded off abruptly at the posterior end, and with a very short flagellum. These stumpy forms are not always to be found, but when present are unmistakable. I found the greatest contrast in the trypanosomes from cerebro-spinal fluid (figs. 30–35), in which the slender and the stumpy forms recall the two types of Trypanosoma dimorphon, and very few intermediate forms were to be found. A count made from four smears of cerebro-spinal fluid gave twenty long and slender forms (43·47 per cent.), eighteen short and stumpy forms (39·12 per cent.), of which one was dividing, and eight intermediate forms (17·39 per cent.). In two preparations, made from the blood of the same monkey (478) on two successive days (pp. 230, 231), I find on one day (figs. 1–5) no stumpy forms, though they were present in smears made the next day (figs. 6–10); and it is noteworthy that in flies fed on this monkey on the first day I obtained no development of the trypanosomes, while the batch of flies fed two days later showed a normal type of infection (p. 231). My best infection of T. gambiense in the tsetse-fly was from a batch of flies fed on monkey 478 on a day (Oct. 19th, p. 237) when it was showing trypanosomes, few in number, but sharply differentiated in form (figs. 11–13).

Castellani (**13**) has stated that T. gambiense, i. e. the trypanosome of Gambia fever, moves with the flagellum forwards, that of the sleeping sickness with the blunt end forwards. I find that the trypanosomes I have observed push their way through blood-corpuscles with the blunt end forwards, but when moving freely they tend to travel with the flagellum forwards.

With due allowance for variations in form and size, I find the structure of T. gambiense very uniform. The nucleus is large and lodged near the middle of the body; it appears to be a compact mass of chromatic granules, without any definite limiting membrane. Near the nucleus a few coarse granulations are commonly seen, which may, however, be few in number or absent. The kinetonucleus is usually close to

the posterior end, but may be a short distance from it when the posterior end is prolonged into a point. The kinetonucleus is usually a minute round body, but may be rod-shaped, a form possibly connected with impending division, though this seems to me doubtful. I observed that in slender forms the kinetonucleus, if rod-shaped, was placed transversely (figs. 12, 30), but in stumpy forms the rod-shaped kinetonucleus was applied to the surface of the body and had a more longitudinal direction (figs. 34, 35). In good preparations it can always be seen clearly that the flagellum does not arise directly from the kinetonucleus, but from a minute granule, which, as stated above, I regard as the true blepharoplast. In some cases the blepharoplast may be very close to the kinetonucleus, or may be over or under it in the preparation, giving the impression that the flagellum arises directly from the kinetonucleus. The marginal flagellum stands well off from the body, forming an undulating membrane of some depth when seen in profile. The distinction between body and undulating membrane is sharp in osmic preparations, but less so in films fixed by drying, owing, I believe, to the deformation and flattening out of the body produced by the latter method. The flagellum takes, in fixed preparations, a variable number of turns, corresponding to pleats in the undulating membrane, which depend probably on the movements of the animal at the time when it was fixed; but as a rule the membrane is least pleated in the extreme forms, both slender and stumpy (compare figs. 5 and 13), most so in the intermediate form. The anterior end of the body is prolonged into a slender filament, running parallel to the flagellum, sometimes nearly to the end of it.

Most previous observers have described and figured T. gambiense as being vacuolated (see especially Bruce and Nabarro [5] and Castellani [13]). It has been regarded as almost a normal feature of the species to have a vacuole near the kinetonucleus.[1] In my experience these vacuolated

[1] "Für besonders charakteristisch wird von einiger Forschern eine Vakuole gehalten, die . . . . nicht immer in gleicher Deutlichkeit hervortritt," Lühe (26), p. 115.

forms are very rare. I have found them only in trypanosomes from the cerebro-spinal fluid, never in those from the blood, and in the former they are by no means universal. I consider the vacuolated condition as an abnormal one, due to the parasite being in unfavourable conditions, or to the reaction upon it of a diseased enfeebled host. Bruce, Nabarro, and Greig (**6**, p. 20) express the view that trypanosomes do not find cerebro-spinal fluid so favourable for growth as blood, and are stunted; they also note (loc. cit., p. 32) post-mortem forms, with large vacuoles, and deformed. Plimmer (**35**) regards the vacuolated form as "probably due to some condition of environment."

Prowazek (**36**) has given three figures of T. gambiense (named by him T. castellanii), all of which differ a good deal from the appearances I am accustomed to see. His first figure (loc. cit., fig. 108) looks like a stumpy form with a vacuole, but it has a long free flagellum, and is perhaps an ordinary form deformed. His second figure (loc. cit., fig. 109) shows a long slender form, evidently in process of division, as *n* and *N* are divided; that being so, one looks at once for the split flagellum which would naturally be found at this stage. The author has figured in the cytoplasm a slender filament which I identify, without hesitation, as the daughter flagellum not traced for its whole length. I think this figure throws some light on the fibrils Prowazek introduces into so many of his figures of trypanosomes. The author's third figure shows a trypanosome "with long, narrow nucleus." Never in all the many trypanosomes of this species that have come under my observation have I seen one with a nucleus such as Prowazek figures in this specimen; I am inclined to regard it as one in which the nucleus has become deformed in the process of smearing.

In short, I can but state that Prowazek's figures represent forms which, in my experience, are aberrant and abnormal; whether this is to be explained by the influence of technique on the parasites, or of a European climate on their hosts, I leave an open question. I am convinced, however, that the appearance, and even the structure, of trypanosomes may be greatly affected by the condition of their hosts.

I am also unable to make head or tail of the "atypical" forms described by Castellani (**13**). I have never seen anything like them. I note that he states that they occur especially in the last stages of disease. As my object was to trace the normal life-history of the parasite, I avoided as much as possible all material in which the parasites were likely to be influenced by a sickly condition of the host. Perhaps that accounts for the great discrepancy between my observations and those of these investigators with regard to the vacuolation of the trypanosome.

(B) The Development of Trypanosoma gambiense in Glossina palpalis and other Diptera.

My observations and experiments on the fate of T. gambiense in invertebrate hosts were carried out chiefly on Glossina palpalis, but I also made a few observations on the species of Stomoxys, common at Entebbe, and on the two common Entebbe mosquitoes, one a species of Tæniorhynchus, the other of Mansonia.

The results obtained with Glossina palpalis were remarkably uniform, and will be briefly summarised before entering into full detail. The trypanosomes at first multiply in the digestive tract of the fly, and by twenty-four hours are found to be differentiated into two types, slender and stout, sharply distinct from one another, with no intermediate forms. The next day, that is, at about forty-eight hours, these two types are succeeded by a more uniform type, so far as structure is concerned, but varying from slender to fairly stout, with all possible transitions, and of considerable length. On the third day after infection trypanosomes are always to be found in the digestive tract, and are forms of great length, relatively, varying from slender to stout, sometimes appearing degenerate in structure and diminished in number, but in other cases numerous, active, and with no signs of degeneration. On the fourth day trypanosomes are very rarely to be found, and, if present, are very scanty in number and of large

size, but are usually absent altogether. In no case have I found any signs of T. gambiense in the tsetse-fly later than the fourth day after infection. Nabarro and Greig (31) found T. gambiense in G. palpalis up to seventy-one hours after feeding; animal trypanosomes up to 100 hours. Throughout these four days of development T. gambiense undergoes a steady and well-marked increase of size.

In the other Diptera I found that T. gambiense went through the same changes of form and structure as in Glossina palpalis. In Stomoxys, however, no trypanosomes were found on the second day (forty-eight hours) after infection. My mosquito experiments are very incomplete, but I found active trypanosomes in Tæniorhynchus as late as seventy-two hours after infection.

In spite of much searching T. gambiense was never found in any organs except those in which digestion of the blood was proceeding; that is to say in the stomach and intestine.

I will now proceed to a more detailed description of my observations.

Preparations made from flies shortly after infection (July 31st, p. 228, and Sept. 8th, p. 229) show the gradual disappearance of the trypanosomes of intermediate type by their conversion partly into slender forms, but chiefly into stout forms. I think it is safe to assume that the intermediate forms with long flagellum become converted into slender forms (compare figs. 41–44), while those with short flagellum become stout forms. There are also many dividing forms found at this period, indicating active multiplication. The process of differentiation is complete twelve hours after infection, and then we have the two sharply marked types, slender and stout, which characterise the first day after infection. Seen in the living condition the slender, twenty-four-hour forms appear transparent, of serpentine appearance, and very active; the stout forms are fish-like or whale-like in form, opaque and granular, and sluggish in movement (figs. 102, 103).

The slender forms (figs. 45, 48, 52, 56–58, 61, 62, and P. R. S., B 78, Pl. XII, figs. 1–6) are distinguished in prepa-

rations, not only by their snake-like form, but also by the clearness of their cytoplasm, which is free from granulations, and remains scarcely, or not at all, tinged by the Romanowsky method of staining. Comparison of earlier stages show that these slender forms arise from the slender or intermediate forms met with in the blood before it is taken up by the fly, by absorption of the granules in the cytoplasm. The whole appearance of these forms suggests the activity and mobility which they are seen to possess when observed in the living state. The flagellum is very long and stands well off from the body, but the undulating membrane is not greatly pleated. The kinetonucleus is a circular dot usually placed at or very near the posterior end, but sometimes at a short distance from it. The nucleus has a dense, compact appearance, and usually a compressed form, but very frequently an irregular outline, with sometimes an appearance as if portions of chromatin were being detached from it, as noted in my preliminary communication (fig. 56, and P. R. S., B 78, Pl. XII, figs. 4, 5, and 6).

The stout forms (figs. 46, 47, 49–51, 53–55, 60, 63–65, 76, and P. R. S., B 78, Pl. XII, figs. 7–14), on the other hand, are large and obese, with cytoplasm coarsely granular and staining deeply. The flagellum is short, the undulating membrane but slightly pleated, and the kinetonucleus, which is circular or rod-shaped, is usually some distance from the posterior end, a point in which they contrast with the "stumpy" forms found in the vertebrate body. Sometimes the posterior end is prolonged into a short "rostrum," a feature which becomes exaggerated at a later stage. The nucleus is large and loose in texture, but of definite outline, often with a peripheral ring of coarser granules, but never showing the appearance of chromatin being ejected, as in the slender forms.

Not only do these two forms of trypanosomes differ in structure and appearance, they also show a curious difference in the mode of division. When the slender forms divide the two daughter-kinetonuclei keep close together at the posterior

end (fig. 217; see also P. R. S., B 78, Pl. XII, fig. 6); but in the stout forms, when the division has reached a certain stage, one of the two daughter-kinetonuclei passes forwards and takes up a position between the two daughter-nuclei, thus producing a characteristic division-stage (compare figs. 54, 55, and P. R. S., B 78, Pl. XII, fig. 12).

On the second day, that is, about forty-eight hours after infection, the trypanosomes are seen to be changing into a type which reaches its perfection on the third day, and is best described from its later development. What I will call the third-day type of trypanosome is of considerable length, appearing under forms both slender and stout, but with transitions between these two variations (figs. 83–96). The body is generally cylindrical, tapering gradually anteriorly and bluntly rounded off posteriorly. The kinetonucleus is round or rod-shaped, sometimes large, and generally situated some distance from the posterior end. The undulating membrane is not much pleated, and the free flagellum is short, even in the more slender forms (fig. 86).

In the preparations of about forty-eight hours we find this type sometimes fully perfected, sometimes only beginning to make its appearance. In one fly I found in the red blood forms similar to those described above as characteristic of twenty-four hours after infection (figs. 76, 77), while the black blood showed forms more advanced towards the third-day type (figs. 73–75). The question at once arises, how does this change of type come about? On account of the uniformity of structure shown by the third-day type I am inclined to derive them all from the stouter type seen at twenty-four hours, and to regard the more slender forms seen on the third day as derived by divisions from the stouter forms. In that case what becomes of the remarkable slender forms seen at twenty-four hours? It would be a tempting hypothesis to suppose that they have conjugated with the stout forms, and that the big forms of forty-eight and seventy-two hours represent zygotes, but I am unable to bring forward any facts in support of this supposition. If the stout twenty-four-hour

forms give rise to all those found later, it is implied that the slender twenty-four-hour forms die off; it is, of course, possible that they may persist and give rise to the later slender forms, and I must confess that my observations do not enable me to decide this point with certainty.

In my smears of flies dissected on the third day, I find great differences in the condition of the trypanosomes. In those from one batch (Oct. 18th, p. 235) the trypanosomes were almost without exception excessively granular and frequently also very vacuolated. They gave me the impression of being degenerate forms, with impaired vitality (figs. 82*a*–88). In another batch (Oct. 22nd, p. 237), however, I found in my smears abundant healthy-looking trypanosomes (figs. 89–92), not vacuolated nor excessively granular, and in the living condition they were extremely active, so much so that I thought I had to do with T. grayi.

In both cases alike, however, no trypanosomes were to be found in the flies dissected the day following.

On the fourth day (ninety-six hours or so) I have very rarely found trypanosomes present in the fly, and only once in smears of this period (Sept. 12th, p. 230). My preparations of them are, unfortunately, very poor, but it is at least possible to trace the form and features of the trypanosomes, which are of a large type, differing in no essential particular from those of the day before (figs. 97–101).

In no case did I ever find T. gambiense in the fly after the fourth day.

The disappearance of T. gambiense from the gut of the tsetse-fly, on or after the fourth day after infection, may mean either that the trypanosomes die out completely, or that they pass into some form which has not been recognised. If they really die out in the fly without completing any life-cycle, it would indicate, in my opinion, that Glossina palpalis is not the true host for this trypanosome, and that some other invertebrate host must be sought for it. I discuss this question more fully below. If, however, the disappearance of the trypanosomes is only apparent, and they really

persist, there are many possibilities which suggest themselves. First, they might pass into some other organs of the fly; against this I may urge that I have repeatedly examined all organs of the fly likely to harbour trypanosomes at various periods after infection, and never found a trypanosome or anything that suggested a stage of a trypanosome, outside the digestive tract. Secondly, they might assume some minute ultra-microscopic form; in that case, however, the function in the life-cycle of such a form would almost certainly be that of infecting a new vertebrate host by inoculation, and every experiment to produce infection with Glossina palpalis more than forty-eight hours after the fly had infected itself gave negative results. A third possibility is suggested to me by my observations on the encystation of T. grayi; it is possible that the trypanosomes which disappeared from the stomach and intestine passed on into the proctodæum in order to become encysted there. It is a matter of the deepest regret to me that I did not make smears of the proctodæum, but the idea of such a possible development of the trypanosomes was not present in my mind when I was in Entebbe; since I never saw T. gambiense in the proctodæum, I did not make any preparations of this region, and so the golden opportunity of deciding this point was lost to me.

Dutton, Todd, and Hannington (15) have published observations on the fate of T. gambiense after being taken up by various Arthropods. In G. palpalis they find "unaltered parasites in the alimentary canal up to forty-eight hours; living but altered trypanosomes up to seventy-two hours after feeding." This does not agree with my experience; I find the trypanosomes beginning to alter in character a few hours after feeding; by alteration, however, the authors mean, apparently, coarse or violent modification of trypanosome-structure. In Stomoxys trypanosomes were found up to twenty hours after feeding. In the larva of Auchmeromyia luteola they were found up to twelve hours after feeding; in Anopheles up to forty-two hours. Trypanosomes were

also obtained in a louse. There is nothing that calls for notice in their observations except the "rounded forms," which they believe to "arise from the englobation of single trypanosomes which have cast off blepharoplast and undulating membrane and become spherical." The authors describe their formation more in detail in the rat flea (louse ?), in which they note "granular, indistinct, obviously degenerating parasites." I regard the rounded form seen by Dutton, Todd, and Hannington as trypanosomes succumbing to, and being digested by, the digestive juices of the alimentary canal, and quite distinct from the rounded forms described by Koch (**20**) and Stuhlmann (**41**), which probably form part of a true developmental cycle. I have not found rounded forms of T. gambiense in any of my preparations.

III. Observations upon Trypanosoma grayi.

My material of this trypanosome is limited, because, for reasons explained above, I did not systematically search for it in Glossina palpalis, so that all the instances of its occurrence that came under my ken were in flies used by me for studying the changes undergone by T. gambiense. In this way I found in all six flies containing T. grayi. Fortunately this trypanosome, when it occurs at all, is found in such vast swarms that a single infected fly furnishes an abundant material. Hence, I have been able to discover some facts of interest relating to this form, notably the process of encystation, not previously observed, so far as I am aware, in any trypanosome, and I have also studied carefully the distribution and occurrence of the many forms of this trypanosome in the different regions of the intestine of the fly; but with regard to this latter point, it is to be regretted that my material is defective, inasmuch as none of my five flies had any trypanosomes in the proventriculus, so that I have had no opportunity of studying the forms occurring in this part of the digestive tract. In spite of much searching I have never found this trypanosome in any organs of the fly other than

the digestive tract (stomach, intestine, and proctodæum). Of the six flies infected by T. grayi that I studied, in one case (Nov. 2nd, p. 238) I did not make any notes, unfortunately, as to the exact provenance of the trypanosomes. With regard to the other five, one (Nov. 10th, 1st fly, p. 241) had the trypanosomes only in the proctodæum. A second fly (Nov. 10th, 2nd fly, p. 241) had evidently not fed recently, and contained only a small quantity of black blood in the intestine, and trypanosomes were found only in the black blood and the proctodæum. The remaining three flies (Nov. 13th, p. 243, Nov. 14th, p. 244, and Oct. 10th, p. 232) showed trypanosomes swarming through the red blood (stomach), black blood (intestine), and proctodæum. In my preliminary account of the encystation (P. R. S., B 79, p. 35) I stated that it was rare to find them in the proctodæum, but I now recognise that this was a mistaken impression on my part; indeed, my limited experience indicates rather that the trypanosomes of this species always occur in the proctodæum, even when they are absent in other parts. But I have never found T. gambiense in the proctodæum.

While I was at Entebbe I had little time to draw and study accurately my preparations, but I made a few drawings of my slides from one fly (Oct. 10th), and some of them were published in the preliminary report by Gray, Tulloch, and myself (P. R. S., B 78, Pl. XIII, figs. 23 and 28). I have since then examined my slides of this fly much more carefully, and found that in this case also the proctodæum contained vast swarms of the trypanosome (p. 233), a fact which I had overlooked at the time of writing my report.

Trypanosoma grayi occurs under such a bewildering variety of forms and sizes that it is a matter of great difficulty at first to see any order or regularity in them. This difficulty is increased by the fact that in one fly the trypanosomes will be found reaching a much larger size, as a whole, than in another; thus, in the flies of Nov. 10th (2), Nov. 14th, and Oct. 10th, some of the trypanosomes are of very large proportions, while in the fly of Nov. 13th they are much smaller and more

slender in build. From my observations I have come to the conclusion that all the different forms may be grouped in three classes, the first of which has many subordinate subdivisions. I distinguish the three classes recognised by me in the following manner: (1) Ordinary or multiplicative forms; (2) slender forms; (3) Herpetomonas forms.

(1) The ordinary forms occur in a variety of sizes due principally to their growth and multiplication. We may distinguish, in the first place, adult or full-grown forms—those of the largest size. These are elongated forms, with the body more or less cylindrical in build; in some the body tapers anteriorly to a fine point (figs. 140, 158, 173, 218, etc.); in others, on the contrary, the body diminishes in thickness very gradually towards the anterior end, up to a point at a short distance from the extremity, from which it narrows rapidly to a stumpy point (figs. 138, 139, etc.). We may conveniently distinguish these two forms as the serpentine and the vermiform respectively; the free flagellum is short in both, but appears longer in the serpentine form, perhaps, in some cases, on account of the difficulty in distinguishing the exact anterior termination of the body. A third variety of the large forms is the tadpole form, in which the posterior end of the body is swollen out (figs. 142, 221, etc., and P. R. S., B 78, Pl. XIII, figs. 23, 26); these are the forms which I formally identified as females, but I am now inclined to regard them simply as full-grown forms which are about to multiply by division.

The process of division, which will shortly be described in detail, leads to a small daughter-individual being split off from the larger parent-individual; in this way young forms are produced (fig. 220, etc.)—the smallest individuals of the ordinary type. These young forms are always present, and frequently very abundant (Nov. 14th, p. 244). Between them and the largest forms every gradation of size is to be found; hence we may safely assume that the small daughter-forms produced by division grow up in time into the large forms, unless they develop into some other type, as will be described presently.

To sum up, we may classify the ordinary type of T. grayi roughly into serpentine, vermiform, tadpole-like, young, and intermediate (i. e. growing) forms. To these, however, must be added a sixth form, the significance of which is not at all clear to me. These are the round forms frequently present, and obviously connected with the young forms. It is easy, when round forms occur, to trace a series of transitional forms between them and the young forms, but whether round forms become young forms, or vice-versâ, it is not so easy to determine. A typical round form has a more or less spherical body, giving off a flagellum, which is entirely free except at its origin (fig. 136). Gray has figured a further development of the round forms, in which the flagellum becomes wrapped round the body in such a way as to simulate a cyst or cuticle in optical section (P. R. S., B 78, Pl. XIV, figs. 44–48), and also forms in which the flagellum appears to be entirely lost (loc. cit., figs. 49–51). I have not found such forms in my preparations, but I have seen those that my colleague has figured. According to Koch (**19**) and Stuhlmann (**41**), such forms are a regular part of the life-cycle, and Robertson (**37**) also describes them in the trypanosome of Pontobdella. Koch describes them as arising by the break-up of large, multinuclear forms, possibly zygotes. Robertson, on the other hand, considers them to be the first form assumed by trypanosomes when taken up from the blood of the vertebrate. Both Koch and Robertson describe the formation of small forms from the round forms.

As round forms are not very frequent in my preparations, I am not able to make any definite statements about them, except that they are connected by transitions with small forms. I am inclined to regard them as a normal temporary resting phase of the small forms, though in a few cases, perhaps, they may be due to imperfect fixation or other deformation due to technique—for instance, alterations during the dissection of the fly before fixation, when owing to any cause the making of smears has been delayed.

In all the different varieties of the ordinary form, as I have

called it, of T. grayi, the structure of the body is in general very uniform, though showing great variations in some points. As regards the cytoplasm, I find remarkable differences, which are evidently due to differences in the action of the stain used (Giemsa's mixture); two types of staining are produced which are well exemplified in the series of dividing forms figured by me (figs. 204–216), all of which are drawn from two preparations made from smears of the same blood, and stained in Giemsa's solution side by side in the same dish. In one preparation the cytoplasm is bluish in colour and shows coarse granulations deeply stained, while the flagella are very faintly stained, appearing sharp and delicate, often difficult to make out clearly (figs. 204, 207, 212, 213, 215, 216). In the other preparation the cytoplasm is reddish in tint and the granulations are scarcely seen, or not at all, while the flagella are deeply stained and appear thick and very distinct (figs. 205, 206, 208–211, 214). The differences in the staining are probably due to differences in the use of the Tannin solution, used to differentiate the stain (see p. 169).

The nucleus (*N*) of T. grayi presents itself as a clump of granules, sometimes compact, oval or round, and in the latter case sometimes showing a distinct rosette-like structure (fig. 182, and P. R. S., B 78, Pl. XIII, figs. 35, 39); more often the nucleus is quite irregular in shape and loose in texture, and in one fly (Nov. 10th, [2], p. 241) the nucleus shows streaks of granules apparently trailing irregularly out from it, in nearly all the large forms (figs. 138–140). This suggests that the coarse granulations of the cytoplasm represent chromidia derived from the nucleus; but they stain slightly differently with Giemsa's stain, the granules of the nucleus having a redder tint, while the chromidial granules are more purple, like the kinetonucleus in tint.

The kinetonucleus (*n*) is large and rod-shaped, its length being about twice its breadth; it is superficial in position, and often appears to bulge from the surface of the body. In very thin smears it is often torn out of the body. It is very

compact and stains deeply, so that no structure can be made out in it.

The most remarkable variations can be seen in the relative positions of *n* and *N*. I distinguish six types[1]: (1) *n* well in front of *N*, with distinct space between (figs. 181, 182); (2) *n* just in front of *N*, with no space between (fig. 156); (3) *n* is at the side of, or overlapping *N* (fig. 175); (4) *n* just behind *N* (fig. 176); (5) *n* far behind *N* (fig. 160); and (6) *n* terminal (fig. 152). Miss E. Y. Thomson has made some counts for me of the different types, which I record below:

		(1)	(2)	(3)	(4)	(5)	(6)
Nov. 13. Red blood	Numbers	1	30	45	47	320	7
(figs. 151–155, p. 243)	Percentage	0·22	6·66	10	10·4	71·11	1·5
Nov. 13. Black blood	Numbers	28	145	73	132	346	—
(figs. 156–164, p. 243)	Percentage	3·8	20	10	18·2	47·7	—
Nov. 13. Proctodæum	Numbers	11	55	33	62	179	5
(figs. 165–170, p. 244)	Percentage	3·1	15·9	9·56	17·9	51·5	1·4
Nov. 10. (2) Black blood	Numbers	113	332	168	65	17	—
(figs. 138–143, p. 241)	Percentage	16·3	47·9	24·1	9·3	2·4	—
Nov. 14. Red blood	Numbers	8	158	108	48	42	—
(figs. 171–173, p. 245)	Percentage	2·19	43·4	29·67	13·18	11·53	—
Nov. 14. Red-black blood	Numbers	40	209	863	314	207	—
(figs. 174–176, p. 245)	Percentage	2·4	12·8	52·8	19·2	12·6	—
Nov. 14. Black blood	Numbers	678	8	155	20	50	—
(figs. 177–182, p. 245)	Percentage	74·4	0·8	17	2·1	5·4	—
Oct. 10. Black blood	Numbers	14	56	49	14	6	—
(figs. 226*a*–231, p. 233)	Percentage	10	40·3	35·2	10	4·3	—
Oct. 10. Red blood	Numbers	25	44	39	3	3	—
(figs. 218–226, p. 232)	Percentage	21·9	38·5	34·2	2·6	2·6	—

These figures show in one fly (Nov. 13th) a great preponderance of forms with *n* posterior to *N*, or even terminal, especially in the red blood; but in all others the forms with *n* in front of or close beside *N* greatly preponderate. (In these statistics no account is taken of the slender and the Herpe-

[1] A convenient graphic notation from these types is as follows:

(1) $\overset{n}{=}\!\!\underset{N}{|}$; (2) $\overset{n}{\underset{N}{|}}$; (3) n/N; (4) $\frac{N}{n}$; (5) $\frac{N}{n}$; (6) $\overset{N}{\underset{n}{|}}$. A type with *n* terminal but anterior could be written $\overset{n}{\underset{N}{|}}$

tomonas-like forms, in which *n* is invariably in front of *N*.) The forms with *n* posterior or terminal are interesting, as it is very probable that they represent very nearly the form under which T. grayi occurs in the vertebrate host; and in this connection their tendency to preponderate in the red blood is significant. I recognise now as erroneous my former statement (P. R. S., B 78, p. 243) to the effect that the forms with *n* posterior were nuper parentes, though this may be so in a few cases (see p. 192). It may be said, however, that for the forms occurring in the intestine of the fly, the normal and typical position of *n* is a little in front of *N*, for not only is this the condition found in those forms (the slender form and the Herpetomonas form presently to be described) in which the position does not vary, but it is also the position which *n* always assumes when division is about to commence.

I have made a detailed study of the process of division in T. grayi, with the object of being able to distinguish clearly between the stages of multiplication and of conjugation. The process of division shows some variations; I will first describe what appears to be the most usual course of events, and after that I will deal with the deviations from this course that are met with.

The first event in the process of division is that the minute basal granule or blepharoplast (see p. 172) of the flagellum divides into two (fig. 151). At the same time the kinetonucleus becomes slightly enlarged and elongated. Starting from the two blepharoplasts the flagellum splits longitudinally and as the two minute blepharoplasts are at first connected by a delicate thread, we obtain a common and characteristic early stage of division, in which, in a trypanosome otherwise of normal aspect, the flagellum shows the appearance of a loop at its proximal end (figs. 204, 205). The splitting of the flagellum continues and the two blepharoplasts travel away from each other, so that the appearance of a loop is lost; but at this stage there is nearly always seen a crossing over of the two halves of the flagellum in the region where it is

split in two (figs. 206, 207). This crossing of the flagella marks a second characteristic stage during which the division of the kinetonucleus takes place. The splitting of the flagellum proceeds a certain distance and then breaks off, so that a longer and a shorter flagellum result from it (figs. 208–210). About the time the splitting of the flagellum is complete the nucleus divides. I have found stages of the division in which a thread or band ran between the two daughter-nuclei, connecting two granules, doubtless of centrosomic nature, imbedded each in the mass of one of the two daughter-nuclei, and difficult to distinguish from the chromatin granules surrounding them (figs. 209, 210). As may be seen from the figures, there is the utmost diversity in the relative positions of the two nuclei and the two kinetonuclei. I must therefore retract my former statement (P. R. S., B 78, p. 243) as to the constant position of n and N after division. When the division of the nucleus and flagellum is complete, the body begins to split (figs. 209–212), starting from the anterior extremity between the two flagella. The body always divides into two unequal portions, giving rise to individuals which are markedly unequal in size and may be distinguished conveniently as parent and daughter respectively. The parent takes the longer flagellum, that is to say, the principle portion of the original flagellum; the daughter takes the shorter flagellum. The two individuals are attached last in the neighbourhood of the kinetonucleus (fig. 212; compare fig. 217), until they finally break loose.

By some authors it has been stated that, in the division of trypanosomes, the daughter flagellum is not split off from the parent, but grows out independently of it. I have paid much attention to this point in T. grayi, and can find nothing to indicate that the new flagellum is formed otherwise than I have stated above, namely, by a process of splitting which starts from the division of the blepharoplast. At the same time I can quite well imagine that in other species the process may take a different course. It is seen that even in T. grayi the splitting does not extend to the whole length

of the parent flagellum. In another species the amount of splitting might be much less, or might not extend beyond the division of the blepharoplast. I do not wish, at present at least, to extend my statements as to the splitting of the flagellum beyond the case which I have studied. That the flagellum does actually split in T. grayi is shown, I think, by the fact that in the early stages the daughter flagellum is distinctly finer and more delicate than the parent flagellum (figs. 205–208). I see no reason why this should be so if the daughter flagellum grew out independently from the blepharoplast.

The process of division described in the foregoing paragraphs is that most usually found, and represents, I believe, the normal course of events. The commonest departure from this course is seen in the division of the flagellum, which may be hastened and be completed before the division of the nucleus (fig. 213), or even before that of the kinetonucleus (fig. 215). This variation shows clearly that the flagellum is independent of the kinetonucleus, a fact which, in my opinion, is a further indication that the kinetonucleus should not be confounded with a true blepharoplast or centrosome.

(2) The slender type (figs. 144, 145, 172, 224–226, etc.), is very uniform in its characters and exhibits but slight variation in contrast with the many forms which I have grouped together as the "ordinary" type. The body is elongated and slender; the cytoplasm is free from coarse granules as a rule, but occasionally a few are seen in the posterior part of the body; *N* is compact, sometimes very compressed; *n* is large, rod-shaped or round, generally filling up the whole width of the body, and invariably in front of *N*; the flagellum is distinct and stains deeply; it stands off but little from the body, forming a very shallow undulating membrane, which is scarcely or not at all pleated, and the free flagellum is very long, and often appears distinctly thickened at its free termination.

The chief variation exhibited by this type is seen in the degree of slenderness of the body; usually slender, with a

compressed nucleus (figs. 225, 226), it is sometimes stouter, with a round or oval nucleus, thus showing an approach to the ordinary young forms (fig. 224, etc.). In one instance (Nov. 10th, 1st fly, p. 241) I have found such moderately slender forms connected by transitions with round forms (figs. 132–137). Only in a single instance have I found what appears to be division; but I am by no means certain that this is not really a case of two trypanosomes accidentally superposed (fig. 147).

The slender type is distinguished in life by its extreme motility. The trypanosomes are seen darting across the field of the microscope in the manner aptly termed "en flèche" by French writers. The body is held stiff and moves with the flagellum, vibrating rapidly in advance. These are the forms termed by Novy (**32**) and by Gray and myself (**29**) "male" forms; I shall consider the point more in detail presently.

As a special development of the slender type should be reckoned, perhaps, the immensely elongated forms seen in figs. 242–244. Unfortunately I have not noted the region in which they occur. A similar form has been figured by Gray (P. R. S., B 78, Pl. XIII, fig. 33). My first impression of these remarkable forms was that they were simply spermatozoa of the fly, but they are connected by transitions with more ordinary forms.

(3) The Herpetomonas-type is to be regarded as a special modification of the slender type, though probably originating directly, in some cases, from ordinary young forms. The H-forms (as I may term them for brevity's sake) are minute and very slender, with the chief bulk of the body behind the kinetonucleus, in front of which the body tapers rapidly to a filamentous prolongation, sometimes of considerable length (figs. 163, 164, 169, 170, 184, 185, 187, 188, etc.). Posteriorly the body is usually bluntly pointed. The nucleus has the form of a clump of granules about the middle of the body. The kinetonucleus is invariably in front of the nucleus. The cytoplasm is clear, but sometimes contains a few coarse

granulations, which frequently occur in front of the kinetonucleus. The undulating membrane is sometimes distinct (fig. 163), but as a rule is rudimentary or absent. The flagellum is very long and very slender and delicate; it stains feebly or not at all, and is often difficult to trace. A typical example of this type is unmistakable, and contrasts sharply in many points with the slender type described above.

The H-forms have been found by me becoming encysted in great numbers in the proctodæum of one of my flies (Nov. 14th, p. 246). I have two smears of this specimen; one of them is a thin smear, the other much thicker. Both the smears show all stages of the encystment in abundance, but in the thin smear the cyst-wall, when developed, is nearly always more or less damaged, being evidently of soft consistence. In the thick smear the cyst-wall is usually intact and perfect, but the finer details of the contained body are not so well shown. Figs. 186–197, 202, 203 are drawn from the thin smear, as were also the figures published by me in my preliminary communication to the Royal Society (P. R. S., B 79, p. 37). Figs. 198–201 are from the thicker smear, which I had not examined at the time of making my preliminary communication.

My preparations of the proctodæum show, in the first place, a certain number of young forms of the ordinary type (figs. 183, 186). Similar young forms are also found as the preponderating type of trypanosome in the hinder part of the intestine of the same fly (p. 245), a fact of interest as indicating that the H-type arises from the young forms of the ordinary type. In the second place the smears show a large number of the H-forms (figs. 187, 188), which are also found sparingly in the hinder part of the intestine. In life they were observed swimming freely, and also occurring in masses attached to the wall of the proctodæum. In the third place there are the various stages of encystment, from the earliest modification of the H-form to the ripe cyst in its final form (figs. 189–202). The cysts at all stages were free in the lumen of the proctodæum, and not attached in any way to its walls.

The first sign of encystment is a shortening of the flagellum, which at the same time appears to become thicker and more distinct, an effect perhaps largely due, however, to its being difficult to distinguish the exact limits of the flagellum from the slender anterior prolongation of the body (compare figs. 189, 192). While the flagellum is becoming retracted the cyst-wall begins to appear round the hinder end of the body (figs. 189–193) in the form of a granular secretion, which stains a dull red with Giemsa's stain. The rate at which the cyst is formed shows great variations relatively to the retractation of the flagellum. In some, with the flagellum still long, an abundance of the cyst secretion is seen (fig. 190). In others, with the flagellum almost completely retracted, the cyst is only just beginning to be formed (fig. 193). The substance of the cyst-wall appears to be made up of distinct masses or grains of the red-staining substance, between which is a more fluid matrix, and appearances are often obtained very similar to those figured by Prowazek for Herpetomonas muscæ-domesticæ (**35***a*). The term "Schleim-cysten," applied by Prowazek to the cysts of Herpetomonas, appears suitable for the present case also, as the frequency with which burst or damaged cysts are met with (figs. 192, 195, etc.) indicates that the substance of the cyst-envelope is of a soft nature.

While the cyst-wall is being secreted the retractation of the flagellum is proceeding, until all that can be seen of it is a round or oval red-staining mass, connected by a red streak with the blepharoplast (figs. 194, 195). The appearances seen at this stage (which is of very common occurrence) are remarkable, and suggest strongly the "flagellar vacuole" described by Leishman as giving rise to the flagellum in the flagellated culture-form of Leishmania. Here, however, the flagellar vacuole, if it may be so termed, is, in its relation to the flagellum, the inverse of that of Leishmania—that is to say, it does not precede its formation but results from its retractation. The flagellar vacuole disappears and only the streak is left (fig. 196); finally the streak fades away too, and

the retractation of the flagellum is complete. Meanwhile the cyst-wall, which made its appearance as a cap round the posterior end of the body, is secreted more and more towards the anterior end, and when the flagellum has completely disappeared, the cyst is formed round the anterior termination of the body, thus producing the characteristic pear-shaped cysts which are very abundant in the preparation (fig. 198). This appears from Prowazek's description to be the final stage of the cyst in the case of Herpetomonas; not so in T. grayi. The cysts, at first pear-shaped, with unlike ends, become more oval, with ends alike or scarcely distinguishable (figs. 199, 200), and finally they become more or less circular in outline, with the wall of even thickness all round (fig. 202). This stage is apparently the ripe cyst, and the last stage that can be observed in the body of the fly.

While the process of encystation is going on, noteworthy changes are taking place in the nuclei. The trophonucleus becomes resolved into chromidia. Usually one large, irregular mass of chromatin can be seen, together with a variable number of irregularly scattered chromatin grains (fig. 197). At the same time the kinetonucleus diminishes in size, apparently also as the result of fragmentation. Amongst the chromatic grains scattered in the body, some can be distinguished by their reddish colour, like that of the nucleus, others by their purple colour, like that of the kinetonucleus (fig. 195); the latter are fewer in number. It is important to note that the cyst substance also stains a reddish tint, similar to that shown by the granules of the nucleus, and it is possible that the disruption of the nucleus is in relation to the secretion of the cyst.

In the ripe cysts the kinetonucleus seems to disappear completely. In some of them it can still be made out plainly (fig. 202). In others it cannot be identified with certainty, but amongst the chromatic grains some can be seen which exhibit a more purple tinge than the others, and which represent probably the kinetonucleus broken up. The significance of these changes could only be made out by studying

the germination of the cyst, and that I have not been able to do.

In a few rare cases I have observed division within the cyst; one such case is figured (fig. 203).

Having, in the foregoing, described the various forms under which I have met with Trypanosoma grayi in Glossina palpalis, I propose now to discuss the significance of the different forms and their relations to one another. This is not an easy matter in the incomplete state of our knowledge of the life-cycle of this trypanosome. Moreover, the forms which T. grayi assumes are so different that they might well be taken for different species were it not that they are all linked by transitions. Taking first the forms which I have grouped together as the "ordinary" type, we may, I think, regard them as the multiplicative form of the trypanosome in the fly, that is to say, the form which, when taken from the vertebrate host, has the function of feeding, growing, and multiplying by division to produce the vast swarms of trypanosomes which are found in infected flies. I base this opinion on the following grounds: First, the large size which this form reaches, a character not likely to be exhibited by forms destined to pass back from the fly to a vertebrate host; such forms are more likely to be of small size; secondly, the frequent occurrence of division and production of young and intermediate forms; thirdly, the great variability of this form, especially in the position of the kinetonucleus. Far from being constantly in front of the nucleus, the kinetonucleus may be behind it or even terminal. From our knowledge of other trypanosomes, I think we may assume that the forms with *n* terminal probably represent most nearly the form under which T. grayi occurs in the vertebrate host. It is true that it is not even known that this trypanosome has a vertebrate host. I have elsewhere (P. R. S., B 79, p. 38) brought forward arguments to favour the belief that T. grayi has a vertebrate host, and is not simply a parasite of tsetse-flies, and in my opinion the occurrence of

a form with *n* terminal, like an ordinary blood-trypanosome, is still more in favour of this belief. I would add, further, that from the conditions under which T. grayi was found to occur in one of my flies (Oct. 10th, p. 232), I am of opinion that the host of T. grayi is a bird, probably some of the numerous species of diving birds which swarm on the shore of the Victoria Nyanza.

We have next to consider the slender forms, formerly regarded by me as male. I do not think this interpretation can be maintained, in view of their distribution and mode of occurrence in the fly. Thus, in one of my flies (Nov. 10th, [1], p. 241), only slender forms, round forms, and H-forms occur, and are confined to the proctodæum (figs. 132–137). Some of them are fairly stout and approach young or ordinary forms in character, but with *n* constantly anterior to *N*. In Nov. 10th (2) also we find most typical slender forms in the proctodæum, but none in the intestine, where ordinary forms of all kinds occur (p. 242, figs. 138–150). In Nov. 13th we find no slender forms in the stomach, but in the intestine and proctodæum we find both slender forms and typical H-forms mixed with ordinary forms (figs. 156–170, p. 243). In Nov. 14th we find slender forms very sparingly in the stomach and intestine, while the proctodæum contains swarms of H-forms and cysts (figs. 171–216, p. 245). Finally, in Oct. 10th we find slender forms present fairly commonly in the stomach and intestine, together with ordinary forms, but in the proctodæum we find immense numbers of the slender and H-forms, without any ordinary forms (figs. 218–233, p. 233).

The data given above show that the primary habitat of the slender type is the proctodæum, of which it may constitute the sole trypanosome-fauna; and further that all other forms may be absent altogether from the fly. This, I think, sufficiently disposes of the notion that the slender form represents the male type. From the proctodæum both the slender form and the H-form may extend forwards; it is noteworthy, however, that the slender form gets further forward, being found in the stomach, while the H-form does not, so far as my

observations extend, get further forward than the hinder part of the intestine. It would be interesting to know if the slender forms penetrate as far as the proventriculus. Unfortunately none of the flies examined by me had trypanosomes in the proventriculus.

The slender form and the H-form are in all probability to be regarded as propagative forms, that is to say, as forms destined to spread the species to other hosts. In favour of this view may be urged their small size and great activity, the absence of multiplication amongst them, their mode of occurrence in the fly, and their constant structure, far removed from that of the ordinary blood-trypanosome and more approaching the Herpetomonas or Crithidia type of structure, that is to say, the type of structure usually associated, in these organisms, with parasitism in the gut of insects. The H-form becomes encysted in the proctodæum, and the cysts are doubtless destined to pass out with the fæces. What is the destiny of the slender type? The manner in which it extends forwards in the gut suggests strongly that it may be destined to infect fresh hosts by the inoculative method.

To sum up my conclusions as to the various forms of Trypanosoma grayi: They may be subdivided at the outset (using Doflein's appropriate terms) into (1) multiplicative forms, varying greatly in size and structure; and (2) propagative forms, very constant in both respects, but of two types. One of the propagative types terminates its lodgment in the fly by becoming encysted in the hind gut; the other is perhaps destined to pass forwards through the proboscis.

If the two types hitherto regarded as male and female be not in reality such, the question arises whether any of the numerous forms of T. grayi are to be regarded as sexual. It is usual, when slender and stout forms of trypanosomes occur together, to interpret them as male and female; but the only final and conclusive proof of such forms being sexual in nature is to find them conjugating. I have spent much time trying to find stages of conjugation in my slides of T. grayi,

but among the many thousands of trypanosomes that have passed under my vision I have found none that could be interpreted with certainty as conjugating forms, and very few which could be suspected of this. Since conjugating forms might conceivably be confused with dividing forms, I studied in detail, and have described above, the process of division and its variations, which are chiefly variations in the rapidity, relative to one another, with which the flagellum and the two nuclei (*n* and *N*) divide. I have found a few specimens which might perhaps be conjugating forms, and have figured three of them. In one (fig. 214) it is seen that the two flagella are widely separate, that there are two large kinetonuclei in close contact, and that there is a single compact nucleus, very dense in texture. If this be a variation of division, it is a remarkable one, not only in the very precocious division of the flagellum, and in the fact that the body has begun to divide before the nucleus has divided, but, above all, in the very large size of the two kinetonuclei; I have never seen any division-stage in which the daughter-kinetonuclei were of such large size. On the other hand, this specimen would very well bear interpretation as a fusion, which had commenced from the hinder end of two individuals, in which the bodies are nearly fused, the nuclei completely so, the kinetonuclei are beginning to unite, and the flagella are still quite separate. If this interpretation be the right one, fig. 216 might possibly represent a further stage of the same process. Another possible instance of conjugation is seen in fig. 143, in which the kinetonuclei are united, while the nuclei are distinct. If these three figures really represent instances of conjugation,[1] they do not throw much light on the characters of the males and females, except to show that one

[1] The forms figured by Stuhlmann (**41**) and interpreted by him as conjugation-stages are very different in appearance, especially in having the two flagella on the outer sides, furthest from each other, instead of close together. Stuhlmann considers the position of the flagella diagnostic of conjugation as compared with division; this makes it still more doubtful if the forms figured by me are really conjugation-stages.

partner has a long free flagellum, the other a short one. I am inclined to identify the two varieties of the ordinary form, termed above the serpentine and the vermiform types, as male and female respectively, and fig. 143 supports this view; but it must be confessed the evidence is meagre. Some of the large ordinary forms (figs. 138, 139) might then be really zygotes.

A few words, in conclusion, upon the differences between T. grayi and T. gambiense and the nature of the former. As a rule the two forms can be distinguished at a glance, whether in the living condition or in preparations. T. gambiense is sluggish in its movements, and when observed in vitro seldom moves out of the field. T. grayi, on the contrary, moves with great rapidity, and is very difficult to keep in the field. The slender forms, in particular, dart across like arrows. But in two flies containing T. gambiense seventy-two hours after infection, I observed that the trypanosomes were very active in their movements (Oct. 22nd, p. 237), and in the preparations very large trypanosomes were found.

The most constant distinctive feature of T. grayi is its large rod-shaped kinetonucleus. In T. gambiense *n* may be round or rod-shaped, but is always much smaller than in T. grayi. Moreover, in T. grayi, though the position of *n* is variable, it is most usually at the side of, or in front of, *N*, and when division is about to take place, *n* is always in front of *N*. In T. gambiense, so far as I have observed, *n* is always behind *N* by at least half the distance between *N* and the posterior end of the body. It is sometimes difficult to draw clear distinctions in words, but I think the figures show that it is impossible to confound T. grayi and T. gambiense at any stage. It is, nevertheless, interesting to note that in the two instances in which T. gambiense did not show any signs of degeneration on the third day in the fly, it made some approach to T. grayi in its appearance and activities. T. grayi, as seen in the fly, is probably a good deal different from the same species in the blood of the vertebrate host.

I think there can be no doubt that the forms described by

Koch (**20**) as developmental stages of T. gambiense were really T. grayi or a similar form.

With regard to the origin of T. grayi, our first notion was, as I have stated, that it was identical with T. gambiense; when this notion was dispelled I was at first inclined to regard it, with Novy, as a parasite of the fly itself, and my discovery of the encystation seemed to confirm this idea, which, however, more mature reflections, especially considerations of the habits of the fly, made me give up. In my preliminary report (P.R.S., B 79, pp. 38, 39) I have stated my reasons for believing that T. grayi has a vertebrate host, and I have nothing to add to them. For various reasons we suspected T. grayi to be an avian trypanosome, though we were not able to prove this, but Novy has shown that it is often impossible to find trypanosomes in bird's blood microscopically when their presence can be demonstrated culturally. The fact that a fly (Oct. 10th, 1905, p. 232), bred in captivity, becomes infected with T. grayi after feeding on fowls used to feed freshly caught flies, seems to me proof positive that T. grayi is an avian parasite[1]; but if so, I may point out as a corollary, it also suggests that T. grayi can be transmitted by the inoculative method.

IV. Remarks on the Life-cycle and Mode of Transmission of Trypanosomes.

The scientific study of the transmission of trypanosomes, and their relation to disease, dates from the publication of Bruce's masterly reports on his investigations upon Nagana in Zululand in 1895 (**3**, **4**). It is not necessary for me to dilate at length upon the results, well known to everyone, of these researches, admirable alike for their conception, execution and presentation, and marking, as Koch has well said, the beginning of one of the most important epochs in the study of the Protozoa.

The publication of Bruce's reports seems to have aroused almost immediately suspicions as to the true nature of sleep-

[1] Koch seems inclined to consider the crocodile as the vertebrate host of T. grayi or of other tsetse-fly trypanosomes.

ing sickness, since Blanchard (1) quotes a sentence from a memoir published in 1898, by J. Brault, in which the opinion is expressed that sleeping sickness is a protozoal disease caused by trypanosomes and transmitted by tsetse-flies. The news, however, of the discovery of trypanosomes in the cerebro-spinal fluid of sleeping-sickness patients, by Castellani in 1903, produced quite a chorus of prophetic utterances, predicting or arguing that sleeping sickness would prove to be transmitted by tsetse-flies. According to Blanchard, this view was expressed by himself on June 18th, by Brumpt on June 27th, and by Sambon on July 1st, 1903, in each case independently of the others. Let who will, however, claim the gift of prophecy or the talent for drawing reasonable inferences from the analogy of established truths, it was nevertheless Bruce, who, in collaboration with his colleagues of the Sleeping Sickness Commission, Nabarro and Greig, first supplied the experimental demonstration of the transmission of sleeping sickness by tsetse-flies. All later observations have but confirmed the statements of these pioneer investigators, without so far adding anything of importance to their results. That Glossina palpalis can and does convey the infection of sleeping sickness, may, I think, be taken as an established fact; and up to the present no other method of infection has been proved to exist. This brings the etiology of sleeping sickness into line with that of other trypanosome infections, all of which, so far as present knowledge extends, are transmitted only by the intermediary of blood-sucking invertebrates, with a single exception—the well-known case, namely, of dourine in horses, a disease known to be transmitted from sick to healthy animals by coitus. This exception to the general rule is of considerable interest, as showing that, in this case at least, the trypanosomes have the power of passing through mucous membranes.

There remains, however, the question of the exact manner in which the infection is transmitted by means of the blood-sucking invertebrate. In considering this question, the facts

known with regard to malaria rise at once to the mind. The researches of Ross, Grassi, and others have demonstrated that in malaria the mosquito is a true host in which the parasite goes through a complicated developmental cycle, at the end of which, and not before, the mosquito is able to infect a fresh vertebrate host with the parasite. A mode of infection of this kind may be termed conveniently a cyclical type of infection, and, since it is effected by the mosquito inoculating healthy subjects with the parasites, we may further characterise it as the inoculative cyclical type. It is an obvious suggestion, from the analogy of malaria, that trypanosomes may also undergo a cycle of development in their invertebrate hosts, a suggestion that received, apparently, concrete proof in the well-known investigations of Schaudinn (**40**) upon Trypanosoma noctuæ. But it is becoming increasingly evident, I think I may say, that Schaudinn's statements with regard to Trypanosoma noctuæ must be regarded with scepticism until they have received confirmation, in view of the many possible sources of error from mixed infections which his material presented. Nevertheless, Schaudinn's results have been regarded by many as conclusive proofs that trypanosomes pass through a developmental cycle in their invertebrate hosts. I am conscious myself of having gone to Uganda to investigate this question with a distinct bias in my mind, believing that the infection of sleeping sickness would prove to be of a similar type to that known in the case of the malarial parasite, and stated by Schaudinn to occur in T. noctuæ—namely, the cyclical inoculative type.

On the other hand, many experimental results, so far obtained, indicate that the mode of transmission of sleeping sickness, and perhaps of other trypanosome infections also, is not of the type of malaria, but is in many cases, at least, a direct one—that is to say, that the trypanosome does not go through a developmental cycle in the invertebrate host, but is inoculated mechanically by the proboscis of the blood-sucking intermediary. Thus Brumpt, in 1903 ([**7**] p. 1497), expressed the view that the rôle of the tsetse-fly could not

be compared to that of Anopheles in malaria, since the tsetse transmits only for forty-eight hours, and no longer. At the present time authoritative opinion on the subject may be said to be divided into two camps, some denying the existence of cyclical infection, others believing it to occur. An extreme example of the former school of thought is Novy, who is of the opinion that the insect plays but "a passive or mechanical part" in the transmission of trypanosomes ([**33**] p. 13 of reprint), and who has cast grave but reasonable doubts upon the correctness of Schaudinn's famous investigations upon Trypanosoma noctuæ.

Confining our attention, for the moment, to the transmission of sleeping sickness, that is to say, of T. gambiense by Glossina palpalis, we have, in the first place, the transmission experiments carried out by Bruce, Nabarro, and Greig (**6**), which can be most conveniently summarised in a tabular form:

Number and reference of experiment.	Number of flies fed on patient.	Flies fed on healthy monkey.	Interval between the two feeds.	Infection produced after—
114 (loc. cit., p. 57) . .	880	530	8 hours	65 days
115 (loc. cit., p. 58) . .	881	509	8 „	65 „
99 (loc. cit., p. 59) . .	582	508	24 „	70 „
97 (loc. cit., p. 60) . .	294	255	24 „	48 „
116 (loc. cit., p. 61) . .	354	267	48 „	65 „

These experiments prove the transmission of the trypanosome of sleeping sickness by G. palpalis, and indicate that the transmission is a direct one, since with a longer interval than forty-eight hours no infection was obtained. I say "indicate," because the fact that freshly-caught flies were used for the experiments invalidates conclusions as to the exact method of the infection, since it was also proved by the same investigators that freshly-caught flies may produce

infection with trypanosomes, without having been fed previously upon infected animals. Thus, in one case a monkey became infected when 216 freshly-caught flies had been fed on it in batches over a period of fifteen days (loc. cit., p. 62); in a second case 894 flies produced an infection after twenty-eight days (loc. cit., p. 62); and in a third case, 759 flies infected after twenty-three days (loc. cit., p. 63). To these three cases may be added two experiments recorded by Greig and Gray (**18**); in the first 980 flies produced an infection after forty-six days (loc. cit., p. 106); in the second, 2299 flies were fed on a monkey over a long period without infecting it. Finally, reference may be made to the result obtained by us (**29**, p. 245), in which a batch of 134 flies fed on a monkey (as a control experiment) produced an infection in it. If we add all these results together we find that a total of 5282 tsetse-flies, freshly caught in the neighbourhood of Entebbe, infected five animals; but if we take separately the experiments of Bruce, Nabarro, and Greig (**6**), made at a time when remedial measures had not been undertaken at Entebbe, a much higher average is the result, since we find that 1869 freshly-caught flies produced three infections, an average of one infection for 623 flies, which is a number very little higher than that of the flies which were operative in the eight-hour transmission experiments. There was, therefore, the possibility that the infections produced in the transmission experiments tabulated above were not really produced by transmission from the infected to the healthy subject, but by flies which had been in an infective condition when first caught, before being used for the experiments. It is noteworthy also how much more quickly, in all the successful experiments, the infection was produced with the freshly-caught flies, so that it would appear to be almost more dangerous to be bitten by free flies in a sleeping sickness locality than by those artificially infected in the laboratory.

A further point to note in the experiments tabulated above is the relatively large number of flies required to produce

infection at the short intervals (eight hours). I believe this is to be accounted for as follows: when a batch of flies has been fed and is put on a second animal eight hours later, the majority of flies being gorged with blood do not feed a second time; the flies that feed at the second opportunity are probably those which did not feed on the first occasion. It is comparatively rare for a fly not to gorge itself the first time it is put on to an animal.[1]

For these reasons we sought to obtain evidence more decisive as to the exact nature of the transmission. As stated in our preliminary report (**29**, p. 244), we took one fly at a time, fed it partly on the infected animal, and then transferred it to the healthy animal. In this way we infected[2] nine out of ten animals each with a single fly, which may, I think, be taken as conclusive proof of the transmission being direct, and not due to any pre-existing infectiveness of the flies used, since the proportion of infective flies amongst those caught while near Entebbe was at that time probably less than one in a thousand. It is true that these experiments were done with the "Jinja" strain of trypanosome (a further proof that it was not due to any previous infection of the flies used in the experiment); but I think it may be claimed that they prove what they were intended to prove—namely, that the tsetse-fly can, and does, effect direct mechanical transference of

[1] I have not made detailed reference to the experiments of Dutton, Todd, and Hannington (**15**), as I wish to confine myself more especially to the region in which I worked. The investigators named seem to have been singularly unsuccessful in their experiments, only four (three with T. gambiense, one with T. dimorphon) giving a positive result out of a total of thirty-nine. It is noteworthy that in three out of their four successful experiments the animal that became infected had been bitten by Glossina fusca as well as G. palpalis. Cazalbou (**14**) seems to have been more fortunate in his experiments with freshly caught G. palpalis. Dutton, Todd, and Hannington consider it "certain that . . . mechanical transmission cannot be the only way in which Trypanosoma gambiense is transmitted from man to man" (loc. cit., p. 213).

[2] Thus confirming a suggestion of Bruce (**4**, p. 3) who wrote, with reference to nagana, "I have no doubt one fly would give the disease if taken while feeding on an affected animal and placed straightway on a healthy one."

trypanosomes from an infected to a clean animal by means of its proboscis.

To prove direct transmission, however, does not disprove the existence of an indirect or cyclical method. It is logically impossible to prove a universal negative since any such proposition is invalidated by a single instance to the contrary. We can only say that our attempts to obtain evidence of a cyclical infection gave negative results. Such attempts were of two kinds: first, experiments to show periodicity in the effectiveness of the flies, such as is known to occur in the case of malaria; secondly, observations on the fate of T. gambiense when taken up by the fly. By experiment we obtained infection up to, but never after, forty-eight hours from the time that the fly fed on the infected animal. By observation, as already stated at length in this memoir, it was found that T. gambiense at first multiplied and became differentiated in the gut of Glossina palpalis or other Diptera, and also increased continually in size, but sooner or later died out and could never be found in G. palpalis on or after the fifth, sometimes not on the fourth day after infection. In all forty-two tsetse-flies have been examined by me at ninety-six hours or later after feeding on infected animals, with invariably negative results, although all the flies from the same batches were found to contain T. gambiense more or less abundantly when dissected at earlier periods.

Very different results from those obtained with sleeping sickness are furnished by the recorded experiments and observations with regard to nagana, the tsetse-fly disease of animals caused by T. brucii. If we take first of all Bruce's experiments on the transmission (3) we are struck at once by the much smaller number of flies used to produce a result. Where hundreds were required for an infection with sleeping sickness, tens or even units were sufficient for an infection with nagana. Thus in one experiment (228, loc. cit., p. 5) eight flies, fed four times on a healthy dog immediately after feeding on an infected one, produced

an infection; the same result was attained in another case (Experiment 222, loc. cit., p. 5) with eight flies after only three feeds; with twenty-four hours' interval (Experiment 291, p. 6), nine flies produced an infection after thirty-eight days, and again, with the same interval (Experiment 232, p. 6), twelve flies infected a dog in the same number of days; with forty-eight hours' interval (Experiment 317, loc. cit., p. 7) seventy flies fed in batches over thirty-one days were required, but longer intervals were not successful. With freshly-caught flies, one hundred and twenty-nine flies, fed in batches, infected a horse after twenty-four days (Experiment 225, loc. cit., p. 15), and ninety-eight flies infected a dog after nineteen days (Experiment 236, p. 16). If these experiments favour the view that the infection is a direct one, they at least indicate that infection of this type is much more easily obtained in this way with T. brucii than with T. gambiense. There is, however, in one of Bruce's experiments a circumstance which, taken in connection with an experimental result of ours, seems to me to show clearly that the infection of T. brucii was not always of the direct type. Bruce's Experiment 225 (loc. cit., p. 15), to which reference has been made already, was carried out to answer the question, "Is the tsetse-fly capable of giving rise to the disease if taken out of the fly country into a healthy locality?" In the account given we read that "the method of carrying out this experiment was to go down to the fly country in the early morning, catch the flies, return to the top of the Ubombo, and straightway place them on the animal under experiment. The greatest care was taken that the flies were caught on a perfectly healthy animal, as to have allowed them to puncture one already affected by the disease would naturally vitiate the experiment." Now in our experiments on direct transmission, already recorded (p. 244), we found that if the fly, after feeding on an infected animal, were fed on two healthy animals in succession, only the first healthy animal became infected, not the second—that is to say, that by puncturing the skin of a healthy animal the

proboscis is "cleaned" for a second one. Hence, if the infection of T. brucii were only by the direct method, the flies caught off a healthy animal, in Bruce's experiment, should have been non-infective. The experiment seems to me, therefore, to indicate that in the case of T. brucii there is infection of a type other than the direct—that is to say, that cyclical infection occurs, doubtless in addition to direct infection.[1]

Turning from experiment to observation, we have the results obtained by Koch (**19**) and Stuhlmann (**41**), who have studied the development of T. brucii in Glossina fusca, and have found a state of things which are in sharp contrast with my results for T. gambiense and G. palpalis.[2]

Koch (**19**) found a multiplication of the trypanosomes, with differentiation into stout and slender forms; next, multinuclear forms, believed to be zygotes, from which arose round forms, which in their turn gave rise to small forms with *n* in front of *N*. Long narrow forms, with *n* far in front of *N*, were also found. In the proboscis fluid trypanosomes were found resembling blood-trypanosomes in form and size, in addition to other forms. Attempts to infect rats with the trypanosomes in the digestive tract of tsetses were always without result. Koch gives reasons for believing that the infection produced in the fly differs according to the state of the trypanosomes in the blood of the sick animal; in flies fed on animals freshly infected, with many trypanosomes in their

[1] The great infectiveness of tsetse-flies in the case of nagana is notorious. According to the unanimous testimony of travellers, confirmed by Bruce's observations, it is only necessary for susceptible animals, such as horses or cattle, to pass through the "fly-belts," in order to acquire the disease, practically as a certainty. This is not the case in sleeping sickness. In Uganda I was struck not so much by the number of sick persons in affected areas as by the number of healthy persons living under precisely the same conditions. The infection of sleeping sickness by the bites of tsetses may be compared to a lottery, in which the prizes are few and the blanks are many.

[2] Bruce (**4**) found, in tsetses infected with nagana, trypanosomes in the stomach up to the end of the fifth day, so long as any blood remained undigested.

blood, development was not obtained, but in flies fed on sickly animals with few trypanosomes in their blood, a vigorous growth and development of the trypanosomes resulted.

Stuhlmann (41) has published a preliminary account of the results obtained by himself and Kudicke. These investigators worked both with freshly-caught flies and with flies bred in captivity. The latter were found to become most easily infected if fed on the infected animal for their first meal after being hatched from the pupa; in 80–90 per cent. of the flies so treated, after a short time (2–4 days) the intestine (Hinterdarm) became full of trypanosomes, indifferent forms showing many division-stages. Stuhlmann's figures of sections show the trypanosomes present in enormous numbers, similar, in my experience, to an infection with T. grayi. From the intestine the infection was found to spread forwards into the stomach (Mitteldarm), but rarely extended further forward than this region, unless the flies were re-fed on healthy animals; when that was done the infection could be traced forward as far as the proventriculus. Infection of the proboscis was not produced in artificially infected flies, but was found in a small percentage of those naturally infected. It was found, moreover, that in artifically infected flies the infection produced in the intestine often gradually died out, and only about 10 per cent. of the experimental flies obtained an infection of the stomach and thoracic intestine; thus the percentage of flies that became thoroughly infected in the laboratory came to about the same as the percentage of flies found infected in nature. The authors figure forms which resemble to a certain extent some forms of T. grayi, but which are characterised by a small round kinetonucleus. They distinguish indifferent forms, long forms, and small forms. The long forms are found in the proventriculus and œsophagus, rarely in the proboscis. The small forms are seen, from the figure given (loc. cit., fig. 158 *a*), to be very similar to the small forms of T. grayi with *n* in front of *N* (fig. 182) except for the characteristic difference of the kinetonucleus. It is remarkable, therefore, that the authors should find the

small forms almost exclusively in the proboscis, seldom in the gut, even in its most anterior parts; for, as I have described above, the corresponding forms of T. grayi are, on the contrary, most abundant in the hindmost parts of the digestive tract. Amœboid forms, with flagella reduced or absent, are also described, and are stated to be present sometimes in great numbers, but only in hungry flies. They are regarded as resting phases, and perhaps correspond to the round forms of T. grayi, but I have never seen anything like the "giant amœboids, with 4–16 nuclei," figured by Stuhlmann (loc. cit., fig. 154).

The following is the scheme of development suggested by Stuhlmann: The infection begins by a multiplication of indifferent forms in the intestine. From this point they spread forwards as far as the proventriculus, where Stuhlmann has seen and figured (loc. cit., fig. 152 *a*—*g*) appearances which he believes to be conjugation. After conjugation, probably, are produced the small forms, which may be the infective forms which pass back into a new vertebrate host. The nature of the long, slender forms is doubtful; they appear simply to die out. In no cases were trypanosomes found in the end gut (proctodæum), nor in the salivary glands, nor in any organs of the fly except the proboscis and the digestive tract proper—that is, as far back as the end of the intestine.

The fact that Stuhlmann and Kudicke used only flies bred in the laboratory for their infection experiments may be taken as proof that the trypanosomes observed by them in these flies were truly stages of T. brucii. Stuhlmann is of opinion, and I agree with him, that trypanosomes are not transmitted by tsetse-flies to their posterity; in other words, that so-called germinative or hereditary infection does not occur.[1] It is evident, then, that T. brucii not only multiplies

[1] I cannot follow Novy (**32**) when he refers to the supposed occurrence of trypanosomes in tsetses that have never fed on animals; he even commits himself to the extraordinary statement that "only a very small percentage of biting insects ever feed on blood" (loc. cit., p. 406). I can only explain such an utterance on the part of an investigator so skilled and experienced by

but goes through a developmental cycle in G. fusca, ending with Herpetomonas-like forms, probably destined to infect vertebrate hosts. These results are entirely different from those obtained by me with T. gambiense in G. palpalis, where I found, without exception, that the trypanosomes did not survive after the fourth day, did not extend forwards into the proventriculus, and did not produce forms with *n* in front of *N*. As compared with T. grayi, the most noteworthy point of the development of T. brucii is that the "propagative" Herpetomonas-like forms occur only in the proboscis or most anterior part of the gut. In T. grayi, on the contrary, they are most abundant in the hindermost part of the gut, where they may become encysted. This difference between the two forms indicates that in T. brucii there is only probably inoculative infection, and no encystment or contaminative infection.

From the results, therefore, both of experiment and observation, it seems to me proved that T. brucii goes through a true developmental cycle in at least one species of the tsetse-flies that are agents in its transmission. Why, then,

his lack of acquaintance with the habits and life-history of tsetse-flies—a knowledge only to be acquired, of course, in Africa. From my own experience, I agree thoroughly with Stuhlmann (**41**, p. 44) that Glossina lives exclusively on blood and contains in its gut only organisms taken up with blood or derived from its mother. Our experience is that Glossina is a most greedy and rapacious bloodsucker; we have seen it feed readily on frogs, lizards, snakes, chameleons, crocodiles (compare Koch [21], p. iv), and birds, as well as mammals. On one occasion, a chameleon put into a fly-cage was at once attacked by them, and, being unable to defend itself, was so bitten that it died in twenty minutes from loss of blood. We also made many attempts to feed tsetses in other ways than with blood, but always without result (compare Stuhlmann, loc. cit., p. 44). On the other hand, there is not the slightest evidence that the tsetse can inherit trypanosomes from its parents (compare Stuhlmann, loc. cit., p. 70). Dutton, Todd, and Hannington (p. 202) hint at this possibility, solely from the far-fetched analogy of Piroplasma in ticks; but in this case it is known that the mother-tick passes on to each of her offspring a supply of undigested blood containing the stages of the parasite. It is not necessary to point out that Glossina differs totally in both habits and life-history from either ticks or mosquitoes.

has T. gambiense given only negative results as regards a developmental cycle? If T. brucii is capable of developing, T. gambiense should be capable of doing so also. I can only explain my repeated failures to obtain development by the hypothesis that G. palpalis is not capable (in Uganda, at least) of acting as the true host of T. gambiense, but only of transmitting it by the direct method. Sleeping sickness, as is well known, has only been introduced into Uganda in comparatively recent times, having apparently come from regions further west. The appalling results produced by the epidemic brought about the appointment of our Commission, which carried on its investigations in Entebbe—that is to say, in a region where great mortality was taking place and the danger was most urgent. But in and around Entebbe G. palpalis is the sole species of tsetse-fly occurring, and has, therefore, obtained the sole credit of transmitting the disease which corresponds with it exactly in distribution. It is very possible that if our Commission had carried on its investigations in a region were more than one species of tsetse occurs, it would have been found that other species were equally effective. Brumpt, in 1904 (**8**), gave reasons for believing G. fusca to be also active in transmitting sleeping sickness.[1] But more decisive on the point is the observation of Koch (**21**), who fed forty-two Glossinæ fuscæ and eleven G. tachinoides, all of them young forms bred in captivity, on rats infected with T. gambiense, with the result that ten to twelve days later eight fuscæ and three tachinoides were found to be infected with trypanosomes that must be identified with T. gambiense, in view of the fact that virgin flies, as we may conveniently call them, were used for the experiment. No such result was ever obtained by me with G. palpalis; and I think it is a sound deduction from the observation of Koch, collated with my own experience, that G. palpalis is

[1] Ross (**38**), however, obtained "entirely negative" results with G. fusca, but acknowledges that there was "not sufficient material in these experiments to come to any definite conclusion."

not a suitable host[1] for the development of T. gambiense, though efficient in transmitting this trypanosome mechanically, while in other species of tsetses T. gambiense is capable of passing through a developmental cycle[2] To find support for this conclusion it is not necessary to rely solely on the analogy of the well-known facts of the transmission of malaria and mosquitoes. The interesting studies of Brumpt (**9, 10, 11**) on the transmission of fish-trypanosomes by leeches have shown that for a given species of trypanosome there is what may be termed a right leech, and that other species of leeches are wrong ones. Thus, for T. granulosum of the eel the right leech, according to Brumpt, is a Hemiclepsis, in which the development is completed, while three others, namely, Calobdella punctata, Hirudo troctina, and Piscicola geometra, were wrong leeches, in which only a part of the development was passed through. There is no reason to suppose that in the case of trypanosomes the same intimate relation between host and parasite does not obtain that is known to occur in the case of other parasites, protozoan or metazoan.

Still less can I adhere to the peculiar view of Novy, that trypanosomes do not go through any life-cycle in their invertebrate hosts, but only a process of multiplication analogous to that seen in artificial cultures. Novy seems to regard the invertebrate host as nothing more, so far as trypanosomes are concerned, than a kind of flying culture-tube, an imitation of art by Nature; and, like some

[1] From the experiments of Bouet (**2**) and Roubaud (**39**) there is evidence for regarding G. palpalis as the true host for T. dimorphon.

[2] In his most recent report Koch (**22**) states that in "Glossinen" (presumably G. palpalis) several species of trypanosomes can be found; one of which, so far found five times, can be identified with T. gambiense. It is not stated, however, in what way its identity is established; to judge from Koch's report, which is far from explicit, the identification was made on morphological grounds, a very unsafe criterion. Koch further states that in two of his five cases the trypanosomes were found in the salivary glands—a state of things contrary to all previous recorded experience (compare Stuhlmann [**41**], p. 23); without further explanation of these statements, further comment is impossible.

other writers, he does not seem to realise the essential distinction between multiplication and development. We can, perhaps, see in this attitude towards the problem the predisposition of an accomplished bacteriologist unaccustomed to think zoologically, if I may use such an expression. He expresses the belief that the trypanosomes found in biting insects are harmless parasites of the fly (p. 406) "derived from plant juices, stagnant waters, etc." (p. 404). In speculating about things unknown it is surely safer to reason from established data than from unfounded hypothesis. At the present time true trypanosomes are only known to occur in the blood of vertebrates, and in the stomachs of insects which suck the blood of vertebrates; hence, it is reasonable to assume that the insects in question obtain their trypanosomes from the vertebrates. When trypanosomes have been found in plant juices or stagnant waters it will be time enough to speculate on the possibility of blood-sucking insects obtaining them from such sources.

Another remarkable fact, to which I would draw special attention, is that trypanosomes, taken up into the digestive tract of the fly, do not infect susceptible hosts if artificially inoculated into them. Bruce ([**4**], p. 5) first discovered this curious fact, and could only infect with T. brucii from the stomachs of tsetses if inoculated not more than half an hour [1] after the fly had infected itself; all inoculations at later periods gave negative results. This has been confirmed by many subsequent investigators of trypanosome development, for example, by Prowazek (**36**), Koch ([**19**], p. 14), Bouet ([**2**], p. 474), Gray and Tulloch (**17**), and ourselves (pp. 227, 234). I agree with the explanation, first suggested I believe by Manson,[2] of this fact, that the trypanosomes

[1] Since infection by the bite of the fly can be obtained up to forty-eight hours, the trypanosomes inoculated by the tsetse must be those which remain in the proboscis and do not pass into the digestive tract. Bruce (**4**) observed trypanosomes of nagana in the proboscis as late as forty-six hours after feeding, though rarely. This agrees with the experimental results.

[2] I am not able to give the exact reference.

in the digestive tract of the fly are developmental, and not merely multiplicative forms, and are not ripe, so to speak, for transference to the vertebrate host. Novy (**32**) considers this to be due to T. gambiense having died out in the flies, but this is certainly not so in our experiment (pp. 227, 234), in which case the fly's intestine contained numerous active T. gambiense. In the latter paper of Novy, MacNeal and Torrey (p. 262) it is stated that "the fact that ingested trypanosomes lose their virulence so rapidly in the stomachs of insects indicates a loss of functional activity, especially of the power of multiplication." This, again, is certainly not the case; multiplication of the trypanosomes proceeds continually in the gut of the insect.

In my opinion the evidence now accumulated (I may mention especially Brumpt [**10**] and Prowazek [**36**] in addition to the authorities already cited) is conclusive in favour both of the general statement, that trypanosomes undergo development, as distinct from multiplication, in invertebrate hosts, and of the more special proposition, that certain species do so in tsetse-flies. I wish now to offer a few suggestions upon the mode of their development and the nature of the invertebrate cycle, as it may be termed. The possibilities of trypanosome development are bound up with the question of their possible or probable phylogeny and course of evolution in the past.[1]

Many authorities have pointed out that trypanosomes may possibly, if not certainly, have two distinct ancestral origins. The first is from a Trypanoplasma-like ancestor in which the anterior flagellum has been lost; this is the "trypanosome with flagellum morphologically posterior"

[1] As I pointed out in the discussion on Hæmoflagellates before the British Medical Association at Exeter, where I have ventilated some of the views here expressed. My phylogenetic speculations were, however, put aside by Sir Patrick Manson, who expressed the view that where a parasite is now found in two hosts, for instance, a vertebrate and an arthropod, it was inherited by both of its hosts from their common ancestor (see 'Lancet,' September 7th,

of Léger (**24**). The second origin is from a Herpetomonas-like ancestor, in which the insertion of the flagellum becomes shifted backwards to form the undulating membrane; Léger's "trypanosome with the flagellum morphologically anterior." Woodcock (**42**) and Lühe (**26**) have gone so far as to base generic distinctions on this alleged difference of origin. For evidence bearing upon evolution in past times the zoologist turns naturally to the documents supplied by the records of development at the present time; and it must be acknowledged that so far there has been no development evidence whatever forthcoming in favour of a Trypanoplasma-like ancestor. No one has yet recorded a developmental stage of a trypanosome with two flagella, not even in those of fishes, which seem most likely to be allied to Trypanoplasma. On the other hand, Herpetomonas forms are common, if not invariable, in trypanosome development, and we may agree with Brumpt (**10**) and Léger (**24**) that in such cases this form certainly represents the ancestral form of these intestinal parasites before they became "sanguicolous." We may therefore look upon the Herpetomonas-form in the development of trypanosomes as a true recapitulative larval form, a type with which zoologists and embryologists are sufficiently familiar in the development of animals of all classes. For the present a Trypanoplasma-like ancestor most remain hypothetical until concrete evidence for it is forthcoming in given cases.

Léger (**24**), confining his speculations to the trypano-

1907, p. 707). As I pointed out at the time, this view offers some difficulties; first, in view of the palæontological facts, showing vertebrates and arthropods to have been distinct in Silurian epochs, if not earlier; secondly, in view of the fact that "Hæmoprotozoa" divide their parasitism sometimes between an arthropod (insect or arachnid) and a vertebrate, sometimes between a leech and a vertebrate. I may point out further that the invertebrate host of a Hæmoprotozoon is always one that sucks the blood of the vertebrate; it remains, therefore, to be explained how the transmission from host to host was effected at the ancestral period when, ex hypothesi, all hosts of a given parasite belonged to a single species.

somes with flagellum morphologically anterior, considers that the Herpetomonas ancestor was "a primitively intestinal or entero-cœlomic [1] parasite of an invertebrate," not necessarily of a blood-sucking one, in which their entire life-cycle took place; that when the invertebrate became blood-sucking in habit, its gut parasites found themselves in a nutrient medium in which they were able to multiply enormously and were thus prepared for life in vertebrate blood, into which they finally succeeded in passing by inoculation through the proboscis. Their primitive habitat is supposed to be shown by the fact that only non-sexual multiplication takes place in the vertebrate, and the invertebrate host is necessary for their sexual development—a statement which, it may be remarked, is by no means definitely proved, however probable, for trypanosomes.

Léger's theory is logical and complete; it only seems to me to present one flaw. If we consider the transmission of trypanosomes generally, we find that it does not always take place by the intermediary of an insect, but may be effected by a leech; and if we include Hæmosporidia, as Léger does in his theory, we then have to reckon with Arachnida as well, in some cases. In other words, the constant trypanosome host is the vertebrate; the inconstant host is the invertebrate. I propose, therefore, to consider the facts from the standpoint of an opposite hypothesis, namely, that the ancestors of trypanosomes were primitively parasites of the gut of vertebrates, like so many flagellates known to exist at present, and that from the gut they passed into the blood of the vertebrate and finally into the gut of the blood-sucking invertebrate. If we attempt to imagine and to reconstruct on this basis the successive stages in the evolution of trypanosome life-cycles, we should probably have the following series of events:

(1) The ancestral form, ex hypothesi, was a flagellate parasitic in the vertebrate gut, which doubtless was

[1] I am not quite sure that I understand the meaning of this adjective, though it has a familiar sound to the morphologist.

disseminated as such parasites are now; it formed resistent cysts in the gut which passed out with the fæces, were scattered abroad, and contaminated the food of fresh vertebrate hosts.

(2) When such a form succeeded in penetrating the intestinal wall and passing into the circulatory system, it found itself in a situation from which there was no escape or outlet by natural channels. Hence, if it did not at once come into relation with blood-sucking invertebrates, it could only have infected new hosts by coming back to the intestine of the vertebrate, becoming encysted there, and passing out with the fæces, as in (1). There is absolutely no evidence that any trypanosomes develop in this way; but a cycle of this kind has been described for Lankesterella ranarum by Hinze whose statements are usually, though perhaps not very logically, considered to be refuted by the observations of Siegel on the Hæmogregarina stepanowi of the tortoise.

(3) Our trypanosome in the vertebrate blood may be supposed to have been taken up sooner or later by a blood-sucking invertebrate, the digestive juices of which it succeeded in resisting. It has acquired now a channel of escape from the vertebrate blood and is no longer obliged to become encysted in the vertebrate gut. Becoming adapted to the invertebrate gut, where it finds the nutriment, namely blood, to which it was accustomed, it now forms in the invertebrate gut the cysts which it formerly produced in the vertebrate. The cysts pass out with the fæces, are spread abroad, and reinfect the vertebrate host by contamination. This is the condition which I believe to be represented by T. grayi, described above; though it is possible that in this case contaminative infection is combined with inoculative, definite proof of either being as yet lacking.

(4) The trypanosome having become thoroughly adapted to the invertebrate gut acquires the power of passing forwards till it reaches its proboscis, and becomes inoculated into the vertebrate host, thus establishing the commonly-occurring inoculative type of infection. Now intestinal cysts become unnecessary and cease to be produced.

It is not necessary for me to dwell upon a possible fifth stage realised in Piroplasma, where the parasite passes through two generations of the invertebrate host. Nothing of the sort is known to occur in the case of trypanosomes.

It is not possible to prove or demonstrate a phylogenetic theory. One can only consider how far it suits known facts or overcomes difficulties. My theory has only one advantage over Léger's—that of explaining away the difference in the invertebrate hosts in different cases. Parasitic flagellates are found in the gut of invertebrates as well as vertebrates. If special stress be laid on the occurrence of Herpetomonas in insects which do not suck blood, such as the house-fly, I may refer to Prowazek's speculations on this form, especially his interesting suggestion that the house-fly is descended from blood-sucking ancestors, which acquired the Herpetomonas from vertebrate blood, so that H. muscæ-domesticæ would represent a stranded and persistent larval stage, comparable to, for instance, the axolotl amongst higher animals.

I have suggested above, and in a former memoir (**28**), the possibility that contaminative infection, the commonest of all methods of infection amongst Protozoan parasites generally, may occur also in the case of trypanosomes infecting vertebrates, basing my suggestion upon the encystation observed by me in T. grayi. Encystation has not been observed in any other species of trypanosome, and with regard to T. brucii, the observation of Stuhlmann (**41**) noted above, that the Herpetomonas-forms are found almost exclusively in the proboscis of the tsetse, rather indicates, as I have already pointed out, that the stage in the life-history which tends to become encysted in the case of T. grayi, does not do so in the case of T. brucii, which would probably be similar, in this respect, to T. gambiense. Moreover, experimental evidence, so far as it exists, is against the occurrence of contaminative infection; in the case of nagana Bruce (**4**) experimented with food and water from localities where the disease is rife, but failed to produce infection with it. Bruce also injected fæces of flies into susceptible animals,

but without result; it is a pity the experiment was not tried of contaminating their food with the fæces. Hence the results to hand of observation and experiment, though they are very incomplete, indicate that the trypanosomes of the pathogenic group, such as T. brucii, belong to stage 4 of my hypothetical phylogeny. Nevertheless, if encystation occurs in one species of trypanosome it may occur in others.

Contaminative infection implies the infection of the vertebrate by way of the digestive tract. It is surprising how often the occurrence of accidental infections of this kind has been noted (see Laveran and Mesnil [**23**]) and yet how seldom it has been the object of direct experiment. Thus Bruce ([**8**], p. 46, Experiment 223) records the case of a dog which became infected with nagana after eating a piece of coagulated blood from the heart of a diseased heifer. It is usual to explain away such infections by supposing that an animal which becomes infected by way of the digestive tract must have had somewhere a lesion through which the trypanosomes penetrated, but this is pure assumption, and from the analogy of Dourine-infection it is quite as feasible to suppose that infection from the digestive tract can take place through the mucous membrane. If the trypanosomes can resist the digestive juices of insects, they may also resist those of vertebrates, and in this connection I may refer to the discovery by Léger (**25**) of an intestinal Trypanoplasma in the fish Box boops. All these facts seem to me to render perfectly possible and even probable the contaminative infection of the vertebrate, by way of the digestive tract, not merely as an exceptional occurrence, but as the normal course in those cases where, as in T. grayi, intestinal cysts are formed by the trypanosome. I may remark that if we start our phylogenetic deductions from Léger's hypothesis, it is not easy to explain the occurrence of encystation in T. grayi, unless we deny to it a vertebrate host altogether—a difficult position, it seems to me, in view of the habits of the fly.

The foregoing arguments may seem to many too specula-

tive and unpractical. Phylogenetic speculations may, however, have a practical value, if only in widening our point of view, and at the same time formulating the possibilities of development, for which the investigator should be prepared. I think the studies of trypanosome development have been too much dominated by preconceived assumptions, and that investigators have too often been biassed by analogies and predisposed to force new facts into old formulæ. It is not necessary to suppose that the development of trypanosomes should be in all cases of the same pattern. If it be true that amongst trypanosomes two quite distinct lines of evolution are comprised, we may expect to find the greatest differences in their mode of development.

In conclusion, I may say that with regard to the main problem of my investigations, namely, the life-cycle of T. gambiense, it is a matter of great regret to me that I have little but negative results to bring forward, and can only offer speculations and hypotheses where I had hoped to have contributed definitely established facts. It was my desire to return to my investigations on this subject, but owing to the lack of the necessary support I have been obliged to abandon the idea. I think now, however, for reasons given above, it would be better to study the etiology of sleeping sickness in regions where it is endemic and where other species of tsetses besides G. palpalis occur. In both these respects Uganda is probably less suitable for the study of the problem than the Congo.

The following propositions summarise briefly the conclusions or personal opinions to which my investigations or reflections have led me.

(1) In Uganda Trypanosoma gambiense begins, but does not complete, a developmental cycle in Glossina palpalis, the method of transmission by this fly in this region being purely mechanical and direct.

(2) In other Diptera also, T. gambiense starts on a development in a precisely similar manner, but without getting so far, or persisting so long, as in Glossina palpalis.

(3) The observations and experiments of Koch, Stuhlmann, and others, show that T. brucii goes through a developmental cycle in G. fusca.

(4) It is probable that T. gambiense has an invertebrate host in which it completes its life-cycle in regions where it is indigenous, and it is possible that the true host may be Glossina fusca.

(5) Considerations of phylogeny indicate that the life-cycles of different trypanosomes should not be expected to be in all cases of one invariable type.

(6) The encystation observed in T. grayi indicates that contaminative infection may occur as well as inoculative.

V. Record of Observations.

The batches of flies used in the experiments here recorded were infected by feeding them once on an infected animal, in all cases a monkey. Those flies which did not feed on the infected animal were destroyed. The flies that had fed were kept alive by being re-fed on a "clean" animal, in almost all cases a guinea-pig, but sometimes a monkey. At first I used to feed the tsetses once every two days, but latterly I found it better to give the flies the chance of feeding daily. The method of feeding in all cases was as follows: The flies were kept in boxes with mosquito-netting at the sides and the box was placed on the skin (previously shaved) of the experimental animal, usually on the belly, so that the flies could feed through the gauze. As a rule they fed readily; indeed, care was necessary in handling the boxes to avoid getting one's fingers bitten through the gauze. The boxes containing the flies were kept over water, as without moisture the flies died quickly. The monkeys used in my experiments were species of Cercopithecus, either pygerythrus or smithi, and in all cases were infected, directly or indirectly, with trypanosomes from human cerebro-spinal fluid. The following

is the record of the infected monkeys used by me, copied from the records of the Sleeping Sickness Commission, and kindly supplied to me by Gray, who entered them in the book.

"Monkey 404.

"June 7th, 1905.—Inject 5 c.c. of cerebro-spinal fluid from case of sleeping sickness, 'Sengoma.' Active trypanosomes are present in this fluid.

"July 16th.—Trypanosomes have not appeared in this animal's blood. Re-inject animal with a few drops of blood from Monkey 420. Trypanosomes are numerous in the blood of this latter animal (see Pl. VIII, figs. 22–25).

"Aug. 2nd.—Trypanosomes have appeared in this animal's blood to-day for the first time.

"Sept. 9th.—Trypanosomes have been regularly present in this animal's blood up to now. To-day the animal was sent to Nairobi at the request of the P. M. O. for the use of Dr. Ross, who is about to conduct some experiments on trypanosome transmission with the local tsetse-flies."

"Monkey 420.

"May 23rd, 1905.—Inject this monkey subcutaneously with 2 c.c. of cerebro-spinal fluid obtained from case of sleeping sickness, 'Vikitikeza.' Trypanosomes are numerous in this fluid.

"June 7th.—Trypanosomes have appeared in this animal's blood to-day for the first time. The parasites are scanty in numbers, and are long and thin for the most part.

"July 13th.—Trypanosomes are numerous in its blood.

"Sept. 18th.—The animal died to-day. Trypanosomes have been constantly present in its blood from the beginning. Post-mortem examination revealed nothing of interest."

"Monkey 478.

"Sept. 14th, 1905.—Inoculate this animal subcutaneously with 15 c.c. of cerebro-spinal fluid from a case of sleeping sickness, 'Sengoma.' Trypanosomes are numerous in the fluid.

"Sept. 26th.—Trypanosomes have appeared in this animal's blood to-day for the first time.

"Oct. 15th.—Trypanosomes numerous. Twelve flies fed on this animal to-day.

"Oct. 19th.—Trypanosomes numerous. Twenty flies fed to-day.

"Nov. 12th.—Trypanosomes numerous. Twenty flies fed to-day.

"Nov. 27th.—Died this morning. Trypanosomes numerous in the blood. One trypanosome seen in a smear of the brain. Glands and spleen somewhat enlarged. Stomach surface shows a few minute black ulcers and several nematode worms. Lungs, heart, etc., are normal."

"Monkey 507.

"Oct. 17th, 1905.—Inject the intestinal contents, with a few drops of normal citrate solution, of one Glossina palpalis subcutaneously into this monkey. This fly had fed on Monkey 478 some forty-six hours previously and was found on dissection to be swarming with trypanosomes (see p. 234).

"Dec. 5th.—This animal's blood has been regularly examined twice a week up to the present, but trypanosomes have never been found."

I begin the systematic record of my observations with the batch of July 31st, 1905. Previous to that date, however, I had made various tentative experiments and observations on the infection of the fly and on the changes of the trypanosomes in it, which I do not propose to record in detail.

Batch of July 31st, 1905.—The object of this experiment was to determine the changes undergone by T. gambi-

ense in G. palpalis during the earliest periods of infection. A number of tsetse-flies freshly caught near Entebbe were fed on Monkey 420. Smears were made at the same time of the monkey's blood. Four of the flies were dissected one hour after feeding, and four more five hours after feeding.

The monkey's blood examined fresh showed trypanosomes fairly abundant, some more slender, active, others stouter, less active. In smears fixed with osmic vapour, well-marked differentiation of slender, stout, and intermediate forms were seen (see P. R. S., B 78, Pl. XII, figs. 16–19). A count gave 45 slender (8 dividing), 48 intermediate (8 dividing), and 3 stout forms.

In the smears from the flies dissected after one hour similar types of trypanosomes were seen, but the differentiation into slender and stout forms appeared to be more pronounced, and fewer intermediate forms were seen (figs. 36–39). The impression given by comparison of the monkey's blood with smears made from the fly at this stage is that the intermediate forms of the former have become converted for the most part into the stouter type of the latter. A count gave 37 slender forms (6 dividing), 13 intermediate forms (2 dividing), and 18 stout forms.

Smears from the flies dissected five hours after feeding showed the differentiation still further advanced, especially of the slender forms, some of which showed the characteristic clear cytoplasm free from granules. Stout forms were also found (figs. 40–44). A count gave 21 slender forms (3 dividing), 6 intermediate forms (1 dividing), and 11 stout forms (1 dividing).

Aug. 1st (twenty-four hours after infection).—Four flies were dissected, but smears were only made of two. The smears, both of the red and black blood, showed sharp differentiation into clear slender and granular stout forms (see P. R. S., B 78, Pl. XII, figs. 1, 2, 10, and 11). A count gave 45 slender (7 dividing), and 13 stout forms (1 dividing).

Aug. 2nd (forty-eight hours after infection).—Three flies

were dissected and smears made of the blood. They showed trypanosomes of elongated, moderately stout form, and others of slender type. Dividing forms were also seen (P. R. S., B 78, Pl. XII, fig. 12).

A remarkable feature of this batch is the excess of slender forms over stout.

Batch of Sept. 8th, 1905.—A batch of flies, caught on the island of Kimmi, were fed on Monkey 420. Four flies were dissected after two hours, three after six hours, and three after twelve hours.

The smears made from the flies dissected after two hours showed slender, stout, and intermediate forms, with many division stages.

In the smears made from flies six hours after feeding, the differentiation of stout and slender forms was more marked. One fly contained Trypanosoma grayi as well as T. gambiense; unfortunately the preparations were a failure.

At twelve hours after feeding the differentiation of the stout and slender forms appeared to be complete.

Sept. 9th (twenty-four hours after infection).—Three flies were dissected and smears made from them. A fourth fly was dissected and the blood examined fresh. In the latter no trypanosomes were seen in the red blood; in the black blood they were found singly and in groups of two or three or even four. The single ones moved rapidly, with flagellum forwards. In the groups they were attached by the posterior ends with the flagella free; individuals thus attached were sometimes unequal in size. A group of three was observed in which four flagella could be distinctly seen, indicating division of one of them. A group of four was noted in which one was much smaller than the others, and one larger; this group separated into two couples; in each couple the trypanosomes were attached tête bêche, the two *nn* opposite and close together (fig. 110). The smears showed trypanosomes differentiated into the normal slender, clear, and stout, granular forms (P. R. S., B 78, Pl. XII, figs. 3–5 and 7–9). Dividing stages of both forms were common.

Sept. 10th (forty-eight hours after infection).—Three flies were dissected; the most successful preparation showed long, moderately stout or slender forms (see P. R. S., Pl. XII, fig. 15); the slender clear forms of the previous day wanting.

Sept. 12th (ninety-six hours after infection).—Three flies were dissected, and smears made of the blood. In the first two flies no trypanosomes were found; in smears of the third trypanosomes were found scantily in the red blood, but fairly abundant in the black. They were, for the most part, remarkable for their large size (p. 183, and Pl. X, figs. 97–101).

Batch of Oct. 1st, 1905.—This batch consisted of ten flies bred from pupæ. They were put on to Monkey 478, which was showing a fair number of T. gambiense in its blood, some smears being made from the monkey at the same time. Seven of the flies fed, three refused.

The smears of the monkey's blood showed trypanosomes chiefly of an indifferent type (figs. 1–5). None of the typical stumpy forms were found, but after some searching a few slender forms (fig. 5) were found. In all 83 indifferent forms, 10 slender, and 1 dividing form were counted in a smear.

Of the seven flies that fed one died before being examined; the remaining six were dissected on Oct. 2nd, 3rd, 4th, 5th, 6th, and 8th respectively. All their organs were examined very carefully, namely the pericardial fluid, Malpighian tubules, salivary glands, proventriculus, stomach, intestine, proctodæum, and in some cases the genitalia. In no case were trypanosomes found of any sort. Some of the flies were full of bacteria.

The negative result obtained in this batch was remarkable and difficult to explain. Two points call for notice—the very slight amount of differentiation in the trypanosomes of the monkey's blood, and the fact that the flies were bred in captivity and probably rather sickly and delicate; but whether the result is to be explained by either of these data cannot be asserted definitely.

Batch of Oct. 3rd.—This consisted of a few flies caught the day previously in the vicinity of Entebbe, and fed on

Monkey 478. The flies were kept in a large cage, with water and vegetation, in a situation where direct sunlight fell on the cage late in the afternoon. Smears of the monkey's blood made the day before (Oct. 2nd) showed a considerable amount of differentiation (figs. 6–10), in contrast with the smears made from the monkey the day previously (Oct. 1st). Stout, slender, and indifferent types could be distinguished; of the first 99, of the second 97, and of the third 34 were counted in one smear. Most of the stouter forms were of the typical stumpy form (figs. 9 and 10). Specially noteworthy is the length of the free flagellum in slender forms, and its shortness in the stout forms (compare figs. 6 and 7); by this character the indifferent forms could also be subdivided into two classes.

It is interesting that the trypanosomes from the blood of the same monkey on two consecutive days should show so little differentiation on one day, so much the next day.

Oct. 4th (twenty-four hours after infection).—Two flies were dissected. In the first a few trypanosomes of the stout type were seen in the black blood. A smear was made in which seven typical stout trypanosomes were counted. In the second fly no trypanosomes were found; the stomach contained a vast number of bacteria.

Oct. 5th (forty-eight hours after infection).—One fly was dissected, and various organs examined. Trypanosomes were found fairly abundantly in the red blood and the black blood. The sucking stomach contained a peculiar clot of blood, in which, however, no trypanosomes were found. The trypanosomes in the fresh condition appeared chiefly of the broad type, but one of more serpentine appearance with pointed posterior end was noted.

The smears made were unfortunately very unsatisfactory as regards preservation. In the best one, of the red blood, a few trypanosomes of moderately stout or moderately slender type (figs. 66–68) were found; in some parts of the smear they occurred in clumps, three or four together. In the smears of the black blood none were found.

Oct. 6th (seventy-two hours).—One fly was dissected; there was no blood in the digestive tract, only some brownish fluid in the intestine, in which two trypanosomes were seen. One smear was made, but no trypanosomes could be found in it.

Batch of Oct. 9th, 1905.—This batch consisted of two flies, both of which had been bred from pupæ in August, and had been kept since then in a fly-cage and fed regularly every two or three days on a fowl. The same fowl had been used for feeding the flies in the breeding-cage—that is to say, flies caught wild near Entebbe and kept in a large cage in order to obtain pupæ from them. The fowl became emaciated and of sickly appearance and died; its place was then taken by a second fowl. Unfortunately the fowl that died was not examined.

On Oct. 9th the two flies were fed on Monkey 409, which was showing a fair number of trypanosomes (T. gambiense) in its blood.

Oct. 10th (twenty-one hours after infection). — One of the flies were dissected. Both stomach and intestine were full of red and black blood respectively, and both were swarming with Trypanosoma grayi. In the proctodæum a few non-motile slender trypanosomes, apparently dead, were seen. No trypanosomes were found in the pericardial fluid, salivary glands, proventriculus, thoracic intestine, or sucking stomach. Examination of smears gave the following results: In all T. grayi was abundant, but prolonged searching was necessary to find T. gambiense, a specimen of which, from this fly, was figured by me (P. R. S., B 78, Pl. XII, fig. 14).

The smears of the red blood showed great variety of forms. The following types could be distinguished: (1) large forms, with hinder end narrow (figs. 218, 219); (2) large forms, with hinder end swollen, apparently about to divide (fig. 221); (3) quite small forms (fig. 220); (4) medium-sized, apparently representing stages in the growth of (3) into (1) and (2); (5) round forms, connected by transitions with (3) (figs. 222,

223); (6) slender forms (figs. 224–226); (7) dividing forms. A count gave the following numbers of each type:

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Numbers .	10	34	7	5	19	26	2
Percentage..	9·7	33	6·79	4·85	18·44	25·24	1·94

The smears of the black blood showed the same types, (1) being perhaps rather more pronounced in its characteristics (figs. 226 *a*–231). A count gave the following results:

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Numbers .	86	31	23	22	3	60	13
Percentage.	36·38	13	9·66	9·24	1·26	25·25	5·46

The two smears made from the proctodæum gave remarkable results. In one after much searching a single, slender form was found. In the other, vast swarms of trypanosomes were found in certain spots; in particular, one huge clump, quite visible to the naked eye, and resembling under a low power a section of spleen or other small-celled tissue. Away from these clumps trypanosomes were scanty. The majority of the trypanosomes were slender, Herpetomonas-like forms (fig. 232), but, unfortunately, badly preserved, having a macerated appearance, with *n* often broken up. A few stouter forms (fig. 233), and some pear-shaped forms without flagellum were also to be found. The fact that very scanty free trypanosomes were seen in the fresh condition indicates that the individuals found in the smears were probably all attached in clumps to the wall of the proctodæum, as noted in the fly of Nov. 14th (p. 245). The fact that the few free trypanosomes seen were non-motile, and that in the smear they appear macerated, indicates that the trypanosomes were moribund for some reason. (I have seen similar results follow from using a dissecting needle, which had been inadvertently dipped into borax carmine.)

Oct. 11th (forty-five hours after infection). The second fly of the batch was dissected; it was found to be very

anæmic, and its digestive tract contained no blood, but swarms of large bacteria. No trypanosomes were found.

Batch of Oct. 15th.—A number of flies, freshly caught near Entebbe, were fed upon Monkey 478.

Oct. 16th.—Four flies (one female, three males) were dissected, at about twenty-one, twenty-four, twenty-seven, and twenty-eight hours after infection respectively. In all of them trypanosomes were found both in the red and black blood, not very abundantly, but slightly more so in the black blood. They were for the most part of stout form, but some slender ones were seen also. In the black blood from the second fly two trypanosomes were seen adhering together by the hinder end; after a time a third added itself to them.

Smears were made from all the flies, both of red and black blood; they showed trypanosomes rather scanty in number, but sharply differentiated into slender and stout types (figs. 45–47); very few dividing forms were seen. In one smear, 33 stout and 4 slender were counted; in another, 88 stout, 4 slender, and 3 dividing.

Oct. 17th.—Five flies were dissected. The first fly (male, forty-five hours after infection) was plump and fairly full of blood, both red and black. Trypanosomes were seen fairly abundant in the red, still more so in the black blood; in the latter many of them were united by the posterior end into groups of as many as five. As these trypanosomes appeared active and healthy, as well as numerous, the experiment was made of injecting some of both red and black blood into a monkey (No. 509), but the injection was without effect, and the monkey never became infected (see p. 227).

Smears of the red blood showed trypanosomes sharply differentiated into stout and slender forms (figs. 76, 77), similar to those found the day previous. Smears of the black blood, however, showed a slightly different type, moderately stout, rather long, and characterised, for the most part, by considerable length of the free flagellum (figs. 73–75).

The second fly had the gut perfectly empty of blood, and contained no trypanosomes.

The third fly (female, forty-seven hours after infection) was gorged with blood. The trypanosomes were not very numerous; some were more slender, others stouter in form; in the red blood a slender one and a stout one were seen hanging on together.

Smears of the red blood showed stout forms, not very numerous, one of which was dividing (fig. 69). Smears of the black blood showed trypanosomes fairly numerous, varying from a moderately slender to a very stout type (figs. 70–72). In one smear were counted 17 slender, 25 stout, 21 intermediate, and 2 dividing forms.

The fourth fly (male, forty-eight hours after infection) was of emaciated appearance; the stomach was full of bacteria and contained no red blood; the intestine contained black blood in which were a few motile trypanosomes. Two smears were made, in each of which a single trypanosome of stout form was found.

The fifth fly (male, fifty hours after infection) had a small amount of red blood, in which no trypanosomes were seen, and a considerable amount of black blood, in which a few active trypanosomes were seen. In the smears, however, no trypanosomes could be found.

Oct. 18th.—Three flies dissected and examined. The first fly (male, sixty-nine hours after infection, not re-fed) showed a small amount of blood in the intestine, black posteriorly, reddish anteriorly. A few motile trypanosomes were seen; none in the proventriculus. In a smear a fair number of trypanosomes were seen, slender or moderately stout in type, some of them very granular; unfortunately the stain was very faint.

The second fly (male, seventy hours after infection, not re-fed) showed a similar condition to the last. A fair number of active trypanosomes were seen in the intestine, some of them long and moderately slender, others broader. Three trypanosomes, originally quite separate, were seen to come together and adhere by their posterior ends. No trypanosomes were found in the proventriculus, proctodæum, or

sucking stomach. The two smears made showed trypanosomes for the most part long and moderately stout (fig. 87), others more slender (fig. 86), and others again stouter (fig. 88), all of them very granular and very different in form and appearance from the two types found at twenty-four hours.

The third fly (female, seventy-two hours, re-fed at fifty-one hours after infection) had the digestive tract gorged with blood, red in the stomach, black in the intestine. No trypanosomes were found in the red blood, but in the black blood as many as three or four in a field (3 mm. apochr. immersion); at first very active, they soon became sluggish under observation. A stout one was seen with very distinct undulating membrane, and with *n* and *N* apparently dividing (fig. 109). Another rather smaller one had the undulating membrane distinct, and the cytoplasm containing refringent granules (fig. 107). A third was long and slender, with groups of refringent granules (fig. 108). Of the red blood five smears were made, and, after much searching, two trypanosomes were found in one of them; one of these was damaged, the other (fig. 82 *a*) was of extremely large size, very granular and vacuolated, and in process of division.

My two smears of the black blood show fairly numerous trypanosomes (figs. 83–85), most of them remarkable for their length and large size, varying in type from slender to stout; they are nearly always very granular and frequently vacuolated. Dividing forms were also seen.

Oct. 19th (ninety-four to ninety-eight hours after infection). —Three flies dissected, two male and one female; the two first had been re-fed Oct. 17th, the last re-fed Oct. 18th. All three flies appeared healthy and full of blood, red and black, but no trypanosomes were found in any of them. Numerous smears were made of each and carefully searched, but no trypanosomes could be found.

Oct. 20th (118–119 hours after infection).—The last two remaining flies (both males) were dissected and examined. No trypanosomes could be found in the digestive tract, neither in the fresh condition nor in smears.

Batch of Oct. 19th, 1905.—A number of freshly-caught Entebbe flies were fed on Monkey 478. At the same time smears were made of the monkey's blood, which showed trypanosomes rather scanty but comprising typical long, slender, and short, stumpy forms, as well as others of more indifferent type (figs. 11–13).

Oct. 20th (twenty-four hours after infection).—One fly dissected. The digestive tract was gorged with blood. Trypanosomes were very scanty in the red blood, more abundant in the black. Smears were made of both, and showed the typical slender and stout forms well differentiated, but rather scanty in number; the stout forms rather in excess.

Oct. 21st (forty-six to fifty hours after infection).—Four flies were dissected (three males, one female). In all of them only black blood was found; in one no trypanosomes were seen, but in the other three flies active trypanosomes of moderately slender type were noted. Smears made showed trypanosomes present, but scanty in number, in all four flies. The trypanosomes were long, varying from moderately slender to moderately stout in type, usually very granular, sometimes vacuolated (figs. 78–80). Dividing forms were seen.

Oct. 22nd.—Three flies were dissected. The first fly (female, seventy hours after infection; re-fed Oct. 21st) had the stomach and intestine very full of red and black blood respectively; the black blood contained a few crystals. Trypanosomes were fairly numerous in the black blood and of slender, active type, similar to T. grayi. One that was watched moved rapidly "en flêche" across the field, and was difficult to keep in view. None were seen in the red blood or in the proventriculus. Smears were made both of red and black blood; in the former no trypanosomes were found. In the smears of the black blood the trypanosomes were not very numerous, but very uniform in type (figs. 93–96). They were all of considerable length, moderately stout or moderately slender. (Their slender appearance in life is perhaps to be taken in a proportionate sense—that is to say, it was their

length which gave the impression of slenderness). Their cytoplasm was not granular or vacuolated, and they appeared perfectly healthy and normal; the free flagellum was relatively short; *n* relatively large, sometimes rod-shaped. No dividing forms were seen.

The second fly (male, seventy-one hours after infection, re-fed Oct. 21st) showed exactly the same condition as the last, and in this case also no trypanosomes were found in the red blood, but in the black blood they were numerous; some more slender in appearance and active in movement, progressing rapidly en flêche; others stouter, slower in movement. Smears were made of the black blood; unfortunately the preparations were not very successful, but they showed trypanosomes similar to those seen in the first fly (figs. 89–92), some very slender.

The third fly (female, seventy-two hours after infection, re-fed Oct. 21st) showed the same condition as the two already mentioned. In the black blood a few trypanosomes were seen, both slender and stout, but less active than those seen in the other two flies. The smears made were defective, but in one of them two trypanosomes of rather stout build were seen.

Oct. 23rd (ninety-four to ninety-eight hours after infection, re-fed Oct. 21st). The five remaining flies were dissected; in all of them both red and black blood was present in the digestive tract; the black blood contained numerous crystals. No trypanosomes were found in the red or black blood, nor in the proventriculus nor proctodæum. Smears were made of the blood, but prolonged searching failed to reveal any trypanosomes.

The remaining eleven flies of this batch were examined at intervals up to Nov. 1st, on which date the last seven were examined; in no case were any trypanosomes found.

Batch of Nov. 1st, 1905.—Some freshly-caught Entebbe flies were fed on Monkey 405, which, however, was showing very few trypanosomes in its blood.

Nov. 2nd.—Some of the flies were dissected; no trypanosomes were found in them with the exception of one, which

was found swarming with T. grayi. Five smears were made, but unfortunately no note was kept as to the parts of the gut from which the smears came—an oversight much to be regretted, as the preparations show trypanosomes of a remarkable type; slender ones varying from small (figs. 235, 236) and medium-sized (fig. 241) to a very great length (figs. 242–244). In some of the very long ones *n* could not be made out clearly, perhaps on account of defective preservation; in others *n* distinct. Large forms also occur (fig. 238), and a form with *N* posterior (fig. 234) is also common. In nearly every case *n* is well in front of *N*; only occasionally *n* at the side, and in two cases noted to be behind *N*. A few dividing forms were seen. In the preparations fixed with absolute alcohol the trypanosomes frequently appeared very granular, and full of chromidia.

Batch of Nov. 3rd, 1905.—A batch of Glossina palpalis, freshly caught, was fed on Monkey 478 (see p. 227), which was showing a fair number of trypanosomes in its blood. Smears were made at the same time of the blood of the monkey. The batch of flies was re-fed on healthy animals on the 5th, 7th, and 8th, and after that daily, until the flies had all been used up for dissection and examination.

The smears of the monkey's blood showed trypanosomes fairly abundant and of various types (see figs. 14–17). Of 212 trypanosomes counted, it is noteworthy that only one was dividing; the remainder comprised the following types of form: stumpy forms (figs. 16, 17), 82 (= 38·1 per cent.); long forms (fig. 14), 63 (= 31·2 per cent.); intermediate forms, slender, 28 (= 12·6 per cent.); intermediate, broad (fig. 15), 38 (= 17·5 per cent.). This shows a slight preponderance of stout over slender types, roughly 4 : 3.

Nov. 4th (twenty-four hours approximately after infection).—Three flies were dissected and examined, and smears made from each. In all of them active trypanosomes were found easily in the black blood, when examined fresh, but a hasty examination of the red blood did not reveal any trypanosomes;

they were found, however, in the smears of both red and black blood. The trypanosomes showed pronounced differentiation into slender and stout types (figs. 48–50), the latter greatly preponderating in number. A small number were seen to be dividing, in nearly all cases stout forms. Examination of seven slides gave the following results:

	Slender.	Stout.	Dividing.
1st fly, red blood	0	11	0
1st fly, red blood	15	64	1
1st fly, black blood	0	23	0
2nd fly, red blood	1	29	0
2nd fly, black blood	10	33	6
3rd fly, red blood	2	7	0
3rd fly, black blood	9	269	4
Total	37	436	11

This gives roughly twelve stout to one slender form.

Nov. 6th (seventy-two hours after infection).—One fly was dissected; the red blood examined fresh showed no trypanosomes, but in the black blood some active trypanosomes were found, chiefly of slender type; one large stout form, however, was seen. Three smears of the black blood were made, but put aside and fixed and stained in England; only on one of them were two trypanosomes found after much searching; both, unfortunately, were badly preserved.

Nov. 7th (ninety-six hours after infection).—Three flies were dissected and all their organs carefully examined for trypanosomes. The flies were apparently healthy and contained both red and black blood. In the first fly (male) a few scarce trypanosomes were noted (by my colleague F. M. Tulloch) in both the red and black blood, but in four smears made (two of each kind of blood) none could be found after prolonged searching. In the second fly (female) one active trypanosome was seen in the black blood, after much searching, but in the smear that was made none could be found. In the third fly (female) no trypanosomes were found.

Nov. 8th (120 hours after infection).—Five flies (four males, one female) were dissected and examined for trypanosomes. In each case the examination was extended to the red and black blood, the proventriculus and proctodæum, and either the testes and seminal vesicles, or the ovaries and the larva (present in one case). No trypanosomes were found.

Nov. 10th (168 hours after infection).—Three flies (all males) were dissected and all organs carefully examined. In one of them nothing was found; the other two were found to be infected with Trypanosoma grayi.

In the first fly the intestine contained a small amount of black blood, in which were corpuscles of normal appearance. No trypanosomes were found in the proventriculus, black blood, testes, or seminal vesicles, but the proctodæum contained slender, very active, and rapidly motile trypanosomes. One smear was made which showed fairly numerous trypanosomes of moderately slender type (figs. 132–135). Round forms (fig. 136) also occur, and others transitional (fig. 137), between the round forms and the prevailing slender type. No dividing forms were seen, nor any encysting forms (for further description see p. 195).

In the second fly also the intestine contained a fair amount of black blood and no red. Trypanosomes were found swarming in the proctodæum and black blood; none were found in the proventriculus, the testes, or the seminal vesicles. Smears were made of the proctodæum and the black blood. The smears of the proctodæum showed an immense number of slender forms with very long flagella, *n* large, transverse, always in front of *N* (figs. 144–146), also a few plumper, shorter forms, with the flagellum short and thick, as if being retracted, as in encysting forms, but without any cyst-wall (figs. 148–150); and, finally, a very few cysts, which, however, always showed the cyst-wall damaged and spread out, probably owing to the thinness of the smear; no dividing forms seen, with one doubtful exception (fig. 147). In the smears of the black blood trypanosomes were also found very abundantly and of types startlingly different from those in

the proctodæum. The trypanosomes exhibited great variation of form, size, and structure, but could be divided into four types: (1) The prevailing type was a large vermiform trypanosome with a very short, free flagellum, the anterior end blunt, and the posterior end narrow and drawn out (figs. 138–139); (2) others had the anterior end more tapering, the posterior more or less swollen (fig. 140); (3) a certain number were broad and fat, as if about to multiply by division (fig. 142); in addition to these three types there were found (4) many small or medium sized, as if resulting from recent division (fig. 141); on the other hand, division stages were extremely rare. In all these forms from the black blood the relative positions of *N* and *N* were extremely variable (for further details see p. 190). A count made from the two best preparations gave the following numbers for the various types:

	1. Vermiform.	2. Tadpole-like.	3. Large, fat.	4. Young, small.	5. Young, medium-sized.	6. Dividing.
1st preparation	80	20	14	65	34	0
2nd preparation	197	152		101	34	5
Total	277	186		166	68	5
Percentage	38·6	26·49		23·64	9·67	0·71

Nov. 11th (192 hours after infection).—One fly (male) was dissected; the intestine contained a small quantity of black blood; the stomach contained no blood but quantities of bacteria. No trypanosomes were found in the black blood, proctodæum, stomach, proventriculus, sucking stomach, testes, or seminal vesicles.

Nov. 12th (216 hours after infection).—Three flies (all males) were dissected; two of them contained red and black blood, one only black; in all three the stomach, intestine, proventriculus proctodæum, and genitalia were examined; no trypanosomes were found.

Nov. 13th (240 hours after infection).—Three flies were dissected. The first fly (male) was gorged with blood, both

red and black; no trypanosomes were found in the red or black blood, the proctodæum, salivary glands, or genitalia. The second fly (male) had the intestine empty except for a small quantity of blackish fluid in the intestine; the stomach was crammed with bacteria; no trypanosomes were found in the stomach, intestine, proctodæum, proventriculus, salivary glands, or genitalia.

The third fly (female) had a small quantity of red blood in the stomach, and the intestine full of black blood; it was found to be swarming with Trypanosoma grayi throughout the stomach, intestine, and proctodæum, but no trypanosomes were found in the salivary glands, larva, or proventriculus; one was found, however, in a teased-up ovary, but this was probably accidental; these excessively motile parasites are often let free during dissection by accidental ruptures of the gut-wall, and swarm out into the salt solution in which the dissection is performed.

Examination of the smears made from this fly gave the following results: In the red blood (figs. 151–155) trypanosomes were excessively abundant, for the most part of moderate size, fairly slender, the free flagellum often very short, sometimes long, and the relative positions of *N* and *n* extraordinarily variable (see p. 190). A count gave the following numbers of different types:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Dividing.
Numbers .	240	12	157	41	9
Percentage .	52·28	2·6	34·2	8·93	1·96

In the black blood also the trypanosomes were very abundant and in the main similar in type to those in the red blood, perhaps rather more slender, more active in appearance (figs. 156–162); but in addition there occurred a small number of slender Herpetomonas-like forms, distinguished at once by their very slender form and transparent appearance; they stain feebly, and also have *n* in front of *N* (figs.

163, 164). A count gave the following numbers of the different types:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Slender, H-like.	Dividing.
1st smear . .	248	90	27	27	103	9
2nd smear . .	258	24	37	13	94	8
Total. . .	506	114	64	40	197	17
Percentage .	53·94	12·15	6·82	4·26	21	1·81

Of the contents of the proctodæum I have two smears; in one, which I may call the pure smear, there are no blood-corpuscles; in the other there is a slight admixture of black blood and the preparation contains blood-corpuscles. The trypanosomes show the same types as in the black blood (figs. 165–170), and a count of the pure smear gave the following results:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Slender, H-like.	Dividing.
Numbers . .	258	62	21	4	106	24
Percentage . .	54·31	13	4·42	0·84	22·31	5·05

Comparison of the figures shows that the percentage of Herpetomonas-like forms is about the same in the black blood and the proctodæum.

Nov. 14th (264 hours after infection).—Three flies (all males) were dissected. In two of them the stomach contained no red blood, but numerous bacteria, and the intestine contained blackish fluid; in both of these the proventriculus, stomach, intestine, protodæum, testes, and seminal vesicles, and in one the salivary glands, were examined without finding any trypanosomes.

In the third fly the digestive tract was found gorged with blood, both red in the stomach and black in the intestine. Trypanosoma grayi was found present in stupendous numbers throughout the stomach, intestine, and proctodæum; in the last named the trypanosomes were found both

free and swimming actively and also adherent to the wall in large patches, resembling, under the low power of the microscope, patches of mould, the whole mass quivering and vibrating with the movements of the flagella. No trypanosomes were found in the testes, seminal vesicles, salivary glands, or proventriculus.

Examination of smears gave the following results: The red blood showed a preponderance of large forms (fig. 173), together with a certain number of small or medium-sized trypanosomes (fig. 171) and a few division stages; slender forms (fig. 172) were also found, but were very scarce. A count gave the following results.

	Large.	Medium.	Small.	Male.	Dividing.
Numbers .	237	64	63	3	14
Percentage .	62·2	16·79	16·5	0·78	3·6

The transitional region between the red and black blood showed a similar state of things, but the trypanosomes were rather smaller, not attaining such great length as in the red blood (figs. 174–176). Division stages were frequent, and a series has been drawn from the two smears of this region (figs. 204–216). No slender forms were found.

The black blood (figs. 177–182) showed in the smears a few large forms, and some of medium size (fig. 177), but the great majority were small forms, like those recently originated from division. In one slide forms were found, which, by their shape—especially the pointed hinder extremity (fig. 178)—approached the Herpetomonas type, but true H-forms were not found. A few round forms also were found. A count resulted as follows:

	Large or medium.	Small.	Round.
Numbers	162	749	23
Percentage	17·34	80·1	2·46

Thus over 80 per cent. were small forms, all of them with *n* in front of *N*, or at the side; none with *n* behind *N*. In

the larger forms the relative position of *n* and *N* varies greatly (see p. 190). Dividing stages were very rare; one is figured (fig. 180).

The smears[1] from the proctodæum showed free forms and all stages of encystment. The free forms show two types: (1) larger, broader forms, resembling the small forms seen in the black blood (figs. 183, 186); (2) slender, Herpetomonas-like forms with narrow bodies pointed posteriorly, and very long flagella, faintly stained (figs. 184, 185, 187, 188). The encysting forms, though forming a continuous series, may be divided into (3) forms with flagellum recognisable (fig. 189–193); (4) pear-shaped with flagellum retracted (figs. 194–198); (5) ripe cysts, more or less spherical (figs. 199–202). A count gave the following results:

	1. Ordinary.	2. H-like.	3. Encysting flagellum present.	4. Flagellum retracted.	5. Ripe cysts.	6. Round forms.	7. Dividing forms.
Number .	41[2]	60	38	62	30	2	1
Percentage.	17·48	25·58	16·22	26·42	12·82	·84	·42

Nov. 15th (288 hours after infection).—Two flies, the last survivors of the batch, were dissected, one a male, the other a female, containing a larva. Both were gorged with blood, red in the stomach, black in the intestine, of both the proventriculus, stomach, intestine, proctodæum, genitalia, and in the female the larva were examined, but no trypanosomes were found.

Stomoxys Experiments.

Nov. 17th.—A batch of Stomoxys sp. fed on Monkey 510, which was showing a fair number of trypanosomes.

Nov. 18th.—Four flies dissected and examined; in all try-

[1] Although the proctodæum contained no blood, my smears show a few blood-corpuscles. I think these must have come from the serum used in making the preparations.

[2] One, and only one, of this number had *n* behind *N*.

panosomes were found in the fresh state. Smears were made and put by for examination. In the smears of two flies no trypanosomes were found; in a third fly they were found in the smears of red blood, not in the black; in the fourth fly they were found both in the red and the black blood. The trypanosomes were slender and stout forms perfectly typical in character (figs. 51–55), the former scarce, and some of the latter dividing. In a smear from one fly 66 stout (6 dividing) were counted, but no slender; in a smear from the other fly 28 stout, of which 11 were dividing, and 4 slender were counted. The high proportion of division-stages is remarkable.

Nov. 19th.—The remaining Stomoxys, 20 in all, were dissected and examined, but no trypanosomes were found in any of them.

Mansonia Experiments.

Nov. 19th.—Two mosquitoes (Mansonia sp.) were fed on Monkey 478.

Nov. 20th.—One was dissected and somewhat hastily examined without any trypanosomes being noted. In the smears, however, trypanosomes were found abundantly, of pronounced slender and stout type (figs. 56–60). In one smear 120 stout and 60 slender were counted; in another, 78 stout, 34 slender; no dividing forms were seen.

Nov. 20th.—One Mansonia was dissected, but no trypanosomes were found, and unfortunately no smears were made.

Tæniorhynchus Experiments.

First batch, Nov. 19th.—Four mosquitoes (Tæniorhynchus sp.) fed on Monkey 478.

Nov. 20th.—Two dissected; in each active trypanosomes were noted. In the smears numerous typical slender and stout forms were found (figs. 61–65). Very few were dividing. In one smear were counted 78 stout, 41 slender forms (of which 4 were dividing); in another smear, 89 stout, 45 slender (1 dividing).

Nov. 21st.—Two dissected; a few rare active trypano-

somes found, others motionless, apparently dead. In the smears made a fair number of trypanosomes were found, mostly of stouter form, often very vacuolated, and with *N* broken up (figs. 81–82); one slender form, apparently much macerated, was found. No dividing forms were seen.

Second batch, Nov. 20th.—A number of Tæniorhynchus fed on Monkey 478. Some were dissected after forty-eight hours (Nov. 22nd); in one a motile trypanosome was seen, but in the smear made from this none were found. The four remaining were dissected after seventy-two hours (Nov. 23rd); the blood had become reduced to a mass of dancing granules, amongst which blood-corpuscles were absent or very rare. In three of the mosquitoes no trypanosomes were found; in the fourth some active trypanosomes of stout type were found, but in the smear made none were found.

The experiments with Stomoxys and mosquitoes were highly incomplete, and it is to be regretted that more were not carried out; but so far as they go they show one interesting result, namely that the trypanosomes become differentiated into exactly the same two forms, stout and slender, as they do in Glossina. Further, the trypanosomes in the digestive tract one day after feeding appeared to have multiplied, as judged both by their number and by the frequent occurrence of dividing forms.

Examination of Lice from Sleeping Sickness Patients.

The Father Superior of the Sleeping Sickness Refuge, conducted by the Algerian White Fathers at Kisubi, kindly caused lice to be collected for me from patients in three degrees of the disease—early, medium, and late. I dissected ten lice of each batch and examined all their organs very carefully. The gut contained in all cases numerous cocci of fair size, in couples or clusters; the hinder part of the intestine frequently contained very large, active, rod-shaped bacilli. No trypanosomes were found in any case.

References.

1. Blanchard, R.—"Sur un Travail de M. le Dr. Brumpt intitulé: quelques faits relatifs a la transmission de la maladie du sommeil par les mouches tsétsé," 'Arch. Parasitol.,' viii, 1904, pp. 573–589.

2. Bouet, G.—"Les Trypanosomiases animales de la Basse-Côte d'Ivoire," 'Ann. Inst. Pasteur,' xxi, 1907, pp. 468–471.

3. Bruce, D.—'Further Report on the Tsetse-Fly Disease or Nagana, in Zululand,' London, 1897, 69 pp., 6 pls.

4. Bruce, D.—'Appendix to Further Report on the Tsetse-Fly Disease, or Nagana, in Zululand,' London, 1903, 21 pp., 1 map.

5. Bruce, D., and Nabarro, D.—"Progress Report on Sleeping Sickness in Uganda," 'Rep. Sleeping Sickness Comm.,' i, No. 2, 1903, pp. 11–88, pls. 1–10.

6. Bruce, D., Nabarro, D., and Greig, E. D. W.—"Further Report on Sleeping Sickness in Uganda," ibid., iv, No. 8, 1903, pp. 3–87, pls. 1–4, 2 maps, 2 text-figs.

7. Brumpt, E.—"Du role des mouches Tsé-tsé en pathologie exotique," 'CR. Soc. Biol., Paris,' lv, 1903, pp. 1496–1498.

8. Brumpt, E.—"Maladie du Sommeil; Distribution Géographique, Étiologie, Prophylaxie," 'CR. Congrès Colon. franç.,' 1904, Sec. Med. et Hyg. colon.,' pp. 25–42.

9. Brumpt, E.—"Sur quelques espèces nouvelles de Trypanosomes parasites des Poissons d'eau douce; leur mode d'évolution," 'CR. Soc. Biol.,' lx, Feb. 2nd, 1906, pp. 160–162.

10. Brumpt, E.—"Mode de transmission et évolution des Trypanosomes des Poissons. Description de quelques espèces de Trypanoplasmes des Poissons d'eau douce. Trypanosome d'un Crapaud africain," 'CR. Soc. Biol.,' lx, 1906, pp. 162–164.

11. Brumpt, E.—"Expériences relatives au mode de transmission des Trypanosomes et des Trypanoplasmes par des Hirudinées," ibid., lxi, July 27th, 1906, pp. 77–79.

12. Brumpt, E.—"Role pathogène et mode de transmission du Trypanosoma inopinatum Ed. et Ét. Sergent. Mode d'inoculation d'autres Trypanosomes," T. c., pp. 167–169.

13. Castellani, A.—"Adult Forms and Developmental Forms of the Trypanosome found in Sleeping Sickness," 'Rep. Sleeping Sickness Comm.,' ii, No. 4, 1903, pp. 9–13, pls. i, ii.

14. Cazalbou, L.—"Expérience d'infection de trypanosomiase par des Glossina palpalis infectées naturellement," 'CR. Ac. Sci., Paris,' cxliii, 1906, pp. 435–437.

15. Dutton, J. E., Todd, J. L., and Hannington, J. W. B.—"Trypanosome Transmission Experiments," 'Ann. Trop. Med. Parasitology,' i, 1907, pp. 201–229.

16. Goldschmidt, R. and Popoff, M.—"Die Karyokinese der Protozoen und der Chromidialapparat der Protozoen- und Metazoenzelle," 'Arch. Protistenkunde,' viii, 1907, pp. 321–343, 6 text-figs.

17. Gray, A. C. H., and Tulloch, F. M. G.—"The Multiplication of Trypanosoma gambiense in the Alimentary Canal of Glossina palpalis," 'Rep. Sleeping Sickness Comm.,' vi, No. 14, 1905, pp. 283–287, 8 figs.

18. Greig, E. D. W., and Gray, A. C. H.—"Continuation Report on Sleeping Sickness in Uganda," T. c. (vi, No. 11), pp. 3–273, 5 pls., 3 maps.

19. Koch, R.—"Vorläufige Mittheilungen über die Ergebnisse einer Forschungsreise nach Ostafrika," 'Deutsch. Med. Wochenschr.,' 1905, No. 47, pp. 1865–69.

20. Koch, R.—"Ueber die Unterscheidung der Trypanosomenarten," 'SB. K. preuss. Akad. Wiss. Berlin,' 1905, No. xlvi, pp. 958–962.

21. Koch, R.—"Ueber den bisherigen Verlauf der deutschen Expedition zur Erforschung der Schlafkrankheit in Ostafrika," 'Deutsch Med. Wochenschr.,' 1906, Sonderbeilage zu No. 51, December 20th, pp. i–viii.

22. Koch, R.—"Bericht über die Tätigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit bis zum 25 November, 1906," ibid., 1907, No. 2, pp. 49–51. (Summary by Mesnil, 'F. Bull. Inst. Pasteur,' v, 1907, pp. 363–365.)

23. Laveran, A., and Mesnil, F.—'Trypanosomes and Trypanosomiases,' translated and much enlarged by D. Nabarro. London, 1907, xix+540 pp., 1 pl., 81 text-figs.

24. Léger, L.—"Sur les affinités de l'Herpetomonas subulata et la Phylogénie des Trypanosomes," 'CR. Soc. Biol.,' Paris, lvii, 1904, pp. 615–617.

25. Léger, L.—"Sur la présence d'un Trypanoplasma intestinal chez les poissons," ibid., lviii, 1905, pp. 511–513.

26. Lühe, M.—"Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten," 'Mense's Handbuch d. Tropenkrankheiten,' iii, 1906, pp. 69–268, pls. vi–viii, 60 text-figs.

27. Minchin, E. A.—"Report on the Anatomy of the Tsetse-fly (Glossina palpalis)," 'Proc. Roy. Soc.,' 76 B, 1905, pp. 531–547, 7 text-figs.; corrected reprint, 'Rep. Sleeping Sickness Comm.,' viii, No. 20, 1907, pp. 106–122, 7 text-figs.

28. Minchin, E. A.—"On the Occurrence of Encystation in Trypanosoma grayi Novy, with Remarks on the Method of Infection in Trypanosomes generally," 'Proc. Roy. Soc.,' 79 B, 1907, pp. 35–40, 8 text-figs.; reprinted 'Rep. Sleeping Sickness Comm.,' viii, No. 22, 1907, pp. 137–142.

29. Minchin, E. A., Gray, A. C. H., and Tulloch, F. M. G.—"Glossina palpalis in its relation to Trypanosoma gambiense and other Trypanosomes," ibid., 78 B, 1906, pp. 242–258, pls. xii–xiv, 11 text-figs., 1 map; reprinted 'Rep. Sleeping Sickness Comm.,' viii, No. 21, 1907, pp. 122–136, 3 pls., etc.

30. Moore, J. E. S., and Breinl, A.—"Note on the Life Cycle of the Parasite of Sleeping Sickness," 'Lancet,' clxxii, May 4th, 1907, pp. 1219–1220, 3 text-figs.

31. Nabarro, D., and Greig, E. D. W.—"Further Observations on the Trypanosomiases (Human and Animal) in Uganda," 'Rep. Sleeping Sickness Comm.,' v, No. 10, 1905, pp. 8–48, 3 pls.

32. Novy, F. G.—"The Trypanosomes of Tsetse-Flies," 'Journ. Infect. Diseases,' iii, 1906, pp. 394–411, pls. xv–xvii.

33. Novy, F. G.—"Trypanosomes," 'Journ. Amer. Med. Assoc.,' xlviii, Jan. 5th and 12th, 1907, 36 pp., 10 figs.

34. Novy, F. G., MacNeal, W. J., and Torrey, H. W.—"The Trypanosomes of Mosquitoes and Other Insects," 'Journ. Infect. Diseases,' iv, 1907, pp. 223–276, pls. vii–xiii.

35. Plimmer, H. G.—"Further Observations on the Effects produced on Rats by the Trypanosomata of Gambia Fever and of Sleeping Sickness," 'Proc. Roy. Soc.,' 79 B, 1907, pp. 95–102, pl. i.

35*a*. Prowazek, S. v.—"Die Entwicklung von Herpetomonas," 'Arb. K. Gesundheitsamte,' Berlin, xx, 1904, pp. 440–452, 7 text-figs.

36. Prowazek, S. v.—"Studien über Säugethiertrypanosomen," ibid., xxii, 1905, pp. 351–395, pls. –16, 4 text-figs.

37. Robertson, M.—"Studies on a Trypanosome found in the Alimentary Canal of Pontobdella muricata," 'Proc. Phys. Soc. Edinburgh,' xvii, 1907, pp. 83–108, pls. iv–vii.

38. Ross, P. H.—"Reports on Experiments to ascertain the Ability of Tsetse-Flies to convey Trypanosoma gambiense from infected to clean Monkeys, and on an Intra-corpuscular Stage of the Trypanosoma,"[1] 'Rep. Sleeping Sickness Comm.,' viii, No. 17, 1907, pp. 80–85.

39. Roubaud, E.—"Transmission de Trypanosoma dimorphon par Glossina palpalis, R. Desb.," 'Ann. Inst. Pasteur,' xxi, 1907, pp. 466–467.

40. Schaudinn, F.—"Generations- und Wirthswechsel bei Trypanosoma und Spirochæte," 'Arb. K. Gesundheitsamte,' Berlin, xx, 1904, pp. 387–439, 20 text-figs.

41. Stuhlmann, F.—"Beiträge zur Kenntniss der Tsetsefliege," 'Arb. K. Gesundheitsamte,' Berlin, xxvi, 1907, pp. 1–83, pls. i–iv, 28 text-figs.

42. Woodcock, H. M.—"The Hæmoflagellates," 'Quart. Journ. Micr. Sci.,' n.s., vol. 50, 1906, pp. 151–331, 65 text-figs.

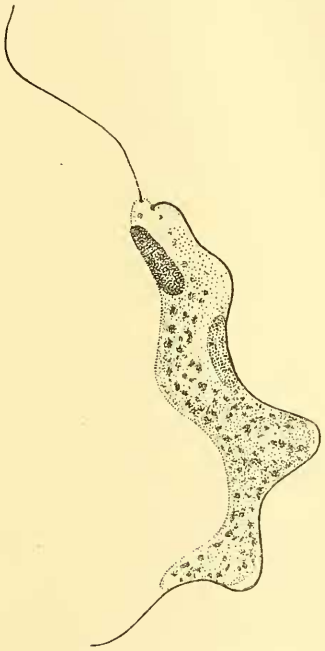
APPENDIX.

Since the above was written a detailed memoir[2] on the cytology of trypanosomes has been published by Salvin-Moore and Breinl, in which the authors take a very different view of the nature of the nuclear apparatus from that taken by me. They regard the kinetonucleus as an "extra-nuclear centrosome" or blepharoplast, comparable with an intra-nuclear centrosome or karyosome contained within the principal nucleus. The extra-nuclear centrosome is described as "a granule or small group of granules which stain like the intra-nuclear centrosome," and the flagellum is stated to arise from one or more of these granules. In development the extra-nuclear centrosome is stated to arise from the intra-nuclear centrosome, which divides into two halves within the nucleus, after which one half is extruded from the nucleus to form the extra-nuclear centrosome.

[1] This title is somewhat misleading; the author states (p. 83), "I have never seen any sign of an intra-corpuscular stage of the trypanosoma."

[2] Salvin-Moore, J. E., and Breinl, A., "The Cytology of the Trypanosomes," Part 1, 'Ann. Trop. Med. Parasitology,' vol. i, pp. 441–480, Pls. xxxviii–xlii.

I am unable to agree with Salvin-Moore and Breinl in regarding the kinetonucleus as of centrosomic nature. On the contrary, I agree with the recently expressed view of Keysselitz, to the effect that "Der Blepharoplast stammt vom Kern ab und besitzt Kernnatur" ('Arch. Protistenkunde,' x,



Text-fig. B.—Trypanoplasma sp. from a pike (Esox lucius), Sutton Broad, Norfolk, to show the kinetonucleus, larger and more darkly stained than the trophonucleus, and the two blepharoplasts, from each of which a flagellum arises; the cytoplasm contains numerouse oarse staining granules. Osmic vapour, Giemsa, × 2000 linear.

p. 129); I should differ from Keysselitz in the use of the word blepharoplast, which, in my opinion, should be used for bodies of centrosomic and not of nuclear nature. I am of opinion, further, that Salvin-Moore and Breinl have confused two distinct structures under the name "extra-nuclear centrosome"; namely, the kinetonucleus, a chromatic body, and the true blepharoplast, a centrosomic or achromatic body. It

is unfortunate from this point of view that these investigators have studied only pathogenic trypanosomes (T. gambiense, brucii, and equinum), in which the kinetonucleus happens to be extremely minute and does not contrast sharply with a blepharoplast; a peculiarity which may be characterised as a "morphological curiosity." If they had studied forms with a large kinetonucleus, like T. grayi, I do not think they would have fallen into this error. Still more decisive against the view of Salvin-Moore and Breinl, it seems to me, is the genus Trypanoplasma (text-fig. B), in which *n* is as large as or even larger than *N*, and in which two distinct blepharoplasts, from each of which a flagellum arises, can be made out plainly in good preparations; pace Salvin-Moore and Breinl, who would not concede, probably, that good preparations can be made anywhere but in Liverpool. Finally, I am unable to agree that a structure of the same nature and reactions as the kinetonucleus exists in the interior of the trophonucleus. Here, again, a form such as T. grayi is very instructive; the large kinetonucleus of this form stains very intensely and of a different tint from the granules composing the nucleus. If a structure similar to the kinetonucleus were contained in the nucleus it would be impossible to overlook it. After what I have said above it is not necessary to point out that for Trypanoplasma the notion that the trophonucleus contains a body similar to the kinetonucleus becomes an absurdity.

The difference, however, between the views of Salvin-Moore and Breinl and of myself is largely one of terminology, since the Liverpool investigators admit that the kinetonucleus arises from the nucleus. They describe it as originating by division of a karyosome, which they compare to the karyosome described by Schaudinn, in Coccidium schubergi; a karyosome of this kind is a chromatic body, even if it contain a centrosome. Further, Salvin-Moore and Breinl regard the two nuclei (*n* and *N*) of a trypanosome as comparable to two differentiated gamete-nuclei, a comparison which I consider far-fetched and misleading in the highest degree, but

which at least shows that they regard both *n* and *N* as bodies of the nature of true nuclei. I hold that the terms centrosome and blepharoplast should not be applied to bodies which are recognised to be of the nature of nuclei; I differ, therefore, from the Liverpool investigators, as from Keysselitz, mainly in a matter of the use of words.

The memoir of Salvin-Moore and Breinl confirms me in the view I have expressed above; namely, that the body of a trypanosome contains two distinct nuclei, and that each of these two nuclei has a centrosomic corpuscle in connection with it; for that, in connection with the trophonucleus, I use the term centrosome simply, since its function is mainly related to division of the nucleus; for that in connection with the kinetonucleus I use the term blepharoplast, since the flagellum takes origin from it. I can imagine that this type of structure may be susceptible of variations and additions; the centrosome might be imbedded in a chromatic mass or true karyosome; the blepharoplast might be lodged in the centre of the kinetonucleus; in either case the essential nature of blepharoplast and centrosome would not be affected.

With reference to my diagram given above (Text-fig. A, p. 174), I should explain that I have purposely given a negative picture, so to speak, of the nucleus and centrosome; that is to say, I have represented the centrosome as a distinct black granule in the midst of colourless chromatic granules making up the trophonucleus; had I represented the centrosome as it really is, namely, as a colourless granule in the midst of deeply staining chromatin-granules, it would have been as difficult to see in my drawing as it usually is in the actual preparations of trypanosomes.

Salvin-Moore and Breinl deny any differentiation of trypanosomes in the blood; they state that the three types, slender, intermediate, and stout, distinguished by me, are "arbitrarily chosen examples in a continuous series of dimensions." To this I reply, first, that it has never been disputed that the different types are connected by transitions, since both the slender and stout forms are differentiations, more or

less strongly marked, of the common intermediate type; secondly, that the difference between the extreme forms is not one of dimensions alone, but of points of structure and morphology which Salvin-Moore and Breinl have overlooked. I am content to let my figures speak for themselves.

Since my work relates almost exclusively to the development of Trypanosoma gambiense in the tsetse-fly, I have no comment to offer on the cycle in the blood, with formation of latent forms, described by Salvin-Moore and Breinl; a discovery of the highest importance, if true, but which does not, in my opinion, disprove the existence of a developmental cycle in an invertebrate host. It is possible, however, that the pathogenic trypanosomes as a group may owe their peculiar properties to having become adapted exclusively to vertebrate hosts; but in that case the problem of transmission and infection becomes difficult to understand. Salvin-Moore and Breinl state (p. 446) that the blood during negative phases, "even if it be properly filtered, is still capable of infecting." This is a very important statement, and I look forward with much interest to the publication by the authors of the evidence on which it is based. At present they have given us none.

Lister Institute, January 15th, 1908.

DESCRIPTION OF PLATES 8—13.

Illustrating Prof. E. A. Minchin's paper on "Investigations on the Development of Trypanosomes in Tsetse-Flies and other Diptera."

All the figures are drawn with the camera lucida to a magnification of 2000 diameters, except Figs. 102–110, Figs. 122–125, and Figs. 127–130 on Pl. 10, which are freehand sketches.

PLATE 8.

Trypanosoma gambiense.

Figs. 1–5.—From the blood of Monkey 478, on October 1st, 1905 (pp. 176 and 230).

FIGS. 6–10.—From the blood of Monkey 478, on October 2nd, 1905 (pp. 176 and 231).

FIGS. 11–13.—From the blood of Monkey 478, on October 19th, 1905 (pp. 176 and 237).

FIGS. 14–17.—From the blood of Monkey 478, on November 3rd, 1905 (p. 239).

FIGS. 18–21.—From the blood of "fresh-fly monkey" (p. 175).

FIGS. 22–25.—From the blood of Monkey 420, on July 16th, 1905 (p. 226).

FIGS. 26–29.—From the blood of male Chimpanzee, eighteen days after inoculation with cerebro-spinal fluid.

FIGS. 30–35.—From human cerebro-spinal fluid.

FIGS 36–39.—From the stomach of Glossina palpalis one hour after feeding on an infected monkey, on July 31st, 1905 (p. 228).

FIGS. 40–44.—From the stomach of G. palpalis five hours after infection on July 31st, 1905 (p. 228).

FIGS. 45–47.—From G. palpalis twenty-four hours after infection, on October 16th, 1905 (p. 234); Fig. 46 from the red blood; Figs. 45 and 47 from the black blood.

FIGS. 48–50.—From G. palpalis twenty-four hours after infection, on November 4th, 1905 (p. 240); red blood from second fly.

FIGS. 51–55.—From Stomoxys sp. twenty-four hours after infection, on November 18th, 1905 (p. 247); Figs. 51 and 52 from one fly; Figs. 53–55 from another.

FIGS. 56–60.—From Mansonia sp. twenty-four hours after infection, on November 20th, 1905 (p. 247).

FIGS. 61–65.—From Tæniorhynchus sp. twenty-four hours after infection, on November 20th, 1905 (p. 247).

PLATE 9.

Trypanosoma gambiense.

FIGS. 66–68.—From Glossina palpalis forty-eight hours after infection, on October 5th, 1905 (p. 231); red blood.

FIGS. 69–72.—From G. palpalis forty-eight hours after infection, on October 17th, 1905, third fly (p. 235); Fig. 60, red blood; Figs. 70–72, black blood.

FIGS. 73–77.—From G. palpalis forty-eight hours after infection, on October 17th, 1905, first fly (p. 234); Figs. 73–75, black blood; Figs. 76, 77, red blood.

FIGS. 78–80.—From G. palpalis forty-eight hours after infection, on October 21st, 1905, first, second, and fourth flies respectively (p. 237).

Figs. 81, 82.—From Tæniorhynchus sp. forty-eight hours after infection, on November 21st, 1905 (p. 248).

Figs. 82 *a*–85.—From Glossina palpalis seventy-two hours after infection, on October 18th, 1905, third fly (p. 236); Fig. 82 *a*, red blood; Figs. 83–85, black blood.

Figs. 86–88.—From G. palpalis seventy hours after infection, on October 18th, 1905, 2nd fly (p. 236).

Figs. 89–92.—From G. palpalis seventy-one hours after infection, on October 22nd, 1905, second fly (p. 238).

PLATE 10.

Trypanosoma gambiense; other parasites of Glossina palpalis.

Figs. 93–96.—From G. palpalis seventy hours after infection, on October 22nd, 1905, first fly (p. 237).

Figs. 97–101.—From G. palpalis ninety-six hours after infection, on September 12th, 1905, third fly (p. 230); Figs. 97, 98, black blood; Figs. 99–101, red blood.

Figs. 102, 103.—Impressions of the two types of trypanosomes seen living in the stomach of G. palpalis twenty-four hours after infection with T. gambiense; sketched July 18th, 1905. (Zeiss. compens. oc. 12, apochr. imm. 3 mm., 1·40 Ap.)

Figs. 104–106.—Impressions of the forms of T. gambiense seen living in G. palpalis forty-eight hours after infection; sketched July 19th, 1905. (Lenses as in last.)

Figs. 107–109.—Impressions of the forms seen living in G. palpalis seventy-two hours after infection, on October 18th, 1905, third fly (p. 236).

Fig. 110.—Sketch to show how a couple of living trypanosomes were attached to one another, on September 9th, 1905 (p. 229).

Figs. 111–125.—Bacteria from the stomach of Glossina palpalis (p. 169); Figs. 111–121, from a preserved smear, × 2000; Figs. 122–125, sketched living.

Figs. 126–131.—Alga-like bodies from the stomach of the fly (p. 170); Fig. 126, from a smear, × 2000; Figs. 127–131, sketches drawn from the living bodies. Between Figs. 127 and 128 the outline of a monkey's blood-corpuscle is sketched for comparison of size.

PLATE 11.

Trypanosoma grayi from Glossina palpalis.

Figs. 132–137.—From November 10th, 1905, first fly, proctodæum (p. 241).

Figs. 138–143.—From November 10th, 1905, second fly, black blood (p. 242).

Figs. 144–150.—From November 10th, 1905, second fly, proctodæum (p. 241).

Figs. 151–155.—From November 13th, 1905, third fly, red blood (p. 243).

Figs. 156–164.—From November 13th, 1905, third fly, black blood (p. 243).

Figs. 165–170.—From November 13th, 1905, third fly, proctodæum (p. 244); all drawn from the "pure" smear.

Fig. 171–173.—From November 14th, 1905, third fly, red blood (p. 245).

Figs. 174–176.—From November 14th, 1905, third fly, transition from red to black blood (p. 245).

Figs. 177–182.—From November 14th, 1905, third fly, black blood (p. 245).

Figs. 183–185.—From November 14th, 1905, third fly, proctodæum (p. 246).

PLATE 12.

Trypanosoma grayi. Stages of encystation and division (except Fig. 217).

Figs. 186–203.—From November 14th, 1905, third fly, proctodæum (p. 246).

Fig. 186.—Ordinary young form.

Figs. 187, 188.—Herpetomonas-like forms, free, not begun to form cysts.

Figs. 189–193.—Forms in which the formation of the cyst and the retractation of the flagelum is proceeding.

Figs. 194–198.—Forms in which the retractation of the flagellum and the formation of the cyst is more advanced. Figs. 194 and 195 show the condition with a flagellum vacuole.

Figs. 199–202.—Ripe cysts. Fig. 202 is from a thin smear and is damaged; the others are from a thick smear. In Figs. 200 and 201, *n* cannot be identified with certainty.

Fig. 203.—Division proceeding within a cyst, which is broken in making the smear.

Figs. 204–216.—From November 14th, 1905, third fly, transitional region between red and black blood (p. 245).

Figs. 204, 205.—Earliest stage of division, the flagellum split at the base.

Figs. 206–208.—The splitting of the flagellum has gone further; in Fig. 207 *n* has divided; in Fig. 208 both *n* and *N* have divided.

Figs. 209, 210.—Splitting of flagellum complete, the two halves of *N* still connected by a band or fibre.

Figs. 211, 212.—Division of the body.

Fig. 213.—Flagellum and *n* divided, *N* still undivided.

Fig. 214.—Flagella distinct, *n* double, *N* single.

Figs. 215, 216.—Flagellum divided, *n* and *N* single.

Fig. 217.—Nearly complete division of a slender form of T. gambiense from stomach of G. palpalis two hours after infection.

PLATE 13.

Trypanosoma grayi from Glossina palpalis.

Figs. 218–226.—From October 10th, 1905, red blood (p. 232).

Figs. 226 *a*–231.—From October 10th, 1905, black blood (p. 233).

Figs. 232, 233.—From October 10th, 1905, proctodæum (p. 233).

Figs. 234–244.—From November 2nd, 1905 (p. 239).